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ADAPTATION TO TRANSPOSITION OF EYE MUSCLES

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The results of Marina's (1915) experiments on transplantation of eye muscles in monkeys have been used to "demonstrate the inadequacy of the traditional theory that function is determined by pre-existing neural pathways" (Fearing, 1930). Marina cut the tendons of the internal and external recti of one eye, crossed the muscles behind the eyeball, and sewed each to the place on the sclera where the other had been inserted. Similar transpositions were made with the superior oblique and external rectus, and with the superior and external recti. Within three to four days after the operation, when the wound had healed, eye movements, whether voluntary or caused by cortical stimulation, or seen in nystagmus, were performed in a normal or nearly normal coördinated manner. Since the muscles were found to adjust themselves to their new situation with such promptness, i.e., muscles now moving the eye out which formerly moved the eye up, etc., Marina concluded that "conduction pathways have no pre-determined function."

All six external ocular muscles are involved in any movement of the eyeball. This is true even for a strictly lateral movement (Duane, 1924). We have therefore repeated certain of Marina's experiments with a view to finding out first, whether coördinated movements after operation might not be the result of the interaction of the undisturbed muscles, and, secondly, if real transference of function does take place, whether there is possibly a brief period of readjustment or learning which would indicate that the original conducting pathways are predetermined, and new ones must be laid down to meet the changed conditions.

Except for operations on three monkeys, we have used dogs and cats as experimental animals. All animals were kept under optimum conditions

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and seemed to experience no appreciable discomfort after the operation. Cats and dogs are provided with four additional external ocular muscles, the retractor bulbi, and Knutsson (1929) has shown that these four muscles contract and relax in the same direction as the corresponding recti. It was therefore necessary to show whether the retractor bulbi were able to rotate the eyball when the other muscles were cut. We were particularly concerned with movements outward and upward.

In a cat we cut the two obliques and all the recti except the inferior rectus of one eye. When the animal had come out of the anesthetic there was no excursion of the operated eye upward or outward as the other eye moved up or in. There was, however, at each shift in position of the normal eye, a slight jerk in the operated eye, usually followed by a wink. Although the retractor bulbi did not produce excursions of the eyeball they did serve to hold the eyeball in place, since the operated eye looked straight forward, in spite of the fact that there was no superior rectus to oppose the action of the inferior rectus.

In another cat the right external rectus had been cut away. Three weeks later there was no squint as the animal looked straight forward (fig. 1, A). When food was held to the right of the animal's head, both eyes were kept in the forward-looking position, and the animal turned the whole head. When the food was placed on the animal's left, both eyes turned left in a normal manner. Coördinated eye reflexes from neck and labyrinth can be especially well demonstrated after a slight dose of sodium amytal. When the cat's head was forcibly turned to the left, the left (normal) eye moved quickly toward the nasal side, then returned slowly to the forward position, while the right eye, on the contrary, remained stationary. When the head was moved to the right, both eyes quickly turned left, then returned slowly to the midline, the right eye lagging behind the left. Four weeks after cutting the external rectus the cerebral cortex was stimulated under light ether anesthesia. Stimulation of the right cortex caused both eyes to turn left, but stimulation of the left cortex caused the left eye only to move. This observation, incidentally, does not agree with that of Russell (1894) who found that after dividing the external rectus of one eye, stimulation of the eye area of the cortex on the opposite side caused the eye to move straight up. We stimulated the left cortex of another cat whose right external and internal recti were cut and again obtained movement only in the normal eye, the operated eye remaining stationary.

In a dog whose right external rectus was detached and then sewed to the upper pole of the eyeball just lateral to the superior rectus, movement of the left eye horizontally to the nose was accompanied by movement of the right eye in a horizontal direction only as far as the midline; the eye then moved up and out at an angle of 60°.

It is evident therefore that the retractor bulbi and the remaining muscles cannot compensate for the lack of the external rectus.

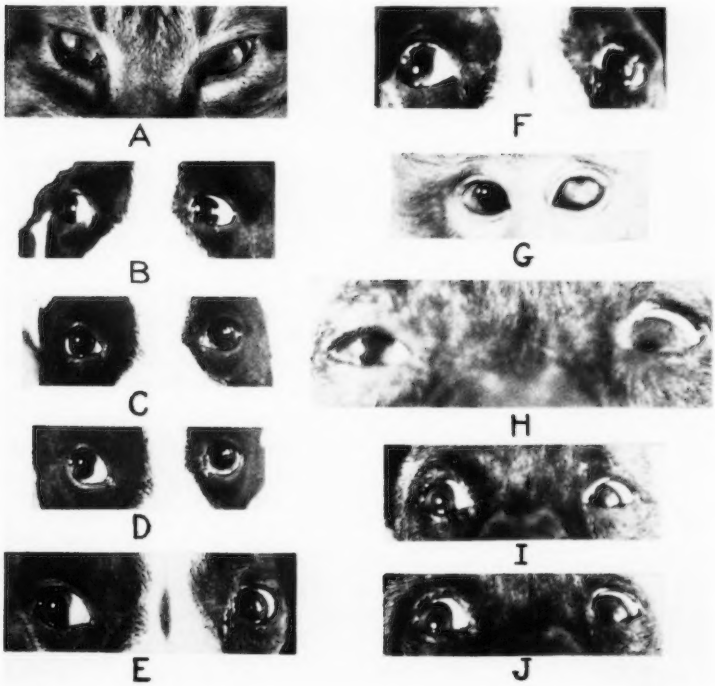

Fig. 1. A. Right external rectus divided 3 weeks; there is no squint. B. Right superior and external recti interchanged 6 days; good lateral movement. C. Right superior and external recti interchanged 6 days; steady gaze forward. D. Right superior and external recti interchanged 6 days; lag in return to forward position after quick movement to right. E. Right superior and external recti interchanged 2½ months; good lateral movement. F. Right superior and external recti interchanged 2½ months; good upward movement. G. Right superior and external recti interchanged. Monkey looking straight forward, showing persisting squint due to slackness in attachment of transposed superior rectus. H. Right superior and external recti interchanged the previous day; photo immediately after removing bandage; right eye looks up instead of out. I. Same dog as in H, 10 days after operation; better coördination in outward movement. J. Same dog as in H, 10 days after operation; quick movement to right still results in upward instead of outward movement.

When the superior rectus was cut in cats, there was again no squint, but eye movements appeared to be normal in all directions. This indi-

cates that evidence of actual transference of function would be more conclusive if the external rectus were to be removed and another muscle which had been sewed in its place were then able to turn the eye outward.

This was successfully accomplished in a number of animals, the superior rectus serving as the transferred muscle. The external rectus was divided as far back as possible and the remaining portion with the tendon was entirely removed. This was to prevent reattachment of the tendon, which had been observed to take place when the tendon only was severed. It was noted that, as certain of these animals began to come out of the anesthetic (amytal) and show nystagmus, the operated eye moved in normally, but there was little outward movement; the eye stopped at the midline. A few days later, movement beyond the midline was observed. When the cortex of these animals was stimulated under light ether anesthesia some weeks after the operation, the eye with the transferred muscle moved outward in each case. In one dog and in one cat it was noticed that under fairly deep anesthesia (ether) the operated eye did not move straight out but moved up as well, approximately 45° from the horizontal. Under very light anesthesia the operated eye now moved more nearly horizontally. At autopsy it was found that in these two animals the superior rectus was not attached quite as far down on the side of the eyball as the scar marking the original insertion of the tendon of the external rectus, but slightly above it, the direction of the pull of the transplanted superior rectus being up as well as out. To produce a strictly lateral outward movement of the operated eye in these animals, joint action of the inferior rectus, the inferior oblique, and the transplanted superior rectus would be necessary. This concerted action was evidently well carried out when these animals were unanesthetized or very slightly anesthetized, but under deeper anesthesia the transplanted superior rectus appeared to be acting alone. This upward and outward action of the transplanted superior rectus was seen in certain other experiments where the operation was a failure in that several strands of the external rectus tendon were still holding at autopsy. Direct stimulation of the superior rectus in these cases caused the eye to move out and up about 30° from the horizontal, and it was found upon dissection that the position of the muscle was such as to make some upward movement inevitable.

Since in all these animals with the external rectus removed outward movement of the eye well past the midline was observed, the superior rectus must have taken over the function of the external rectus. To produce coördinated movements of the eyes there must have been readjustment in the central nervous system, since now the superior rectus of the operated eye was acting in the same direction as the internal rectus of the normal eye, and antagonistically to the internal rectus of the operated eye.

In a series of animals we successfully transposed the superior and external recti. We usually detached the superior rectus first and sewed it to the insertion of the external rectus. The external rectus was then cut loose, brought over the superior rectus and attached to the upper pole of the eyeball. The muscles were gently freed as far back as possible in order that the pull upon contraction would be more nearly in a straight line. At autopsy it was always found that one muscle crossed over the other toward the posterior half of the eyeball. The direction of pull of the transferred superior rectus was sometimes purely lateral, though more often there was some upward movement as well. The external rectus was always found to pull slightly out as well as up. Eye movements three to four days after the operation seemed to be normal as Marina had found, for at this time there were no strictly false movements, i.e., movements in the wrong direction, but it was often observed that the extent of movement out and up was not so great as in the normal eye and there was often a lag (fig. 1, B–F).

To follow the course of transference of function from the earliest possible moment we stimulated the cortex of several animals immediately after transposing the muscles. The operated eye moved up and only slightly out in each case. In one cat the operated eye gave slight jerks only as the normal eye moved in toward the nose. It was found that the superior rectus had been rather tightly stretched to make the attachment at the proper point on the eyeball, and the resulting tension was too great to allow the eye free movement. The superior rectus was then detached and the brain again stimulated. The operated eye now moved nearly straight up, the direction being only some 15° away from the vertical, while the normal eye made the usual horizontal movement toward the nose. As one would expect, therefore, stimulation of the opposite cortex immediately after transference of the muscles causes the external rectus to contract no matter where it is attached.

The eye movements made by one monkey as it came out of the anesthetic were especially instructive. This monkey had been brought to the laboratory blind in the left eye due to corneal opacity. Both eyes, however, moved in a coördinated manner in all directions. The external rectus of the right eye was in this case attached to the top of the eye, and the superior rectus brought down over it to be attached at the side. As the monkey began to come out of the anesthetic (ether) there was nystagmus; when the left eye moved inward horizontally, the right eye moved up and out at an angle of 60°; and when the left eye moved out the right eye moved in to a greater degree than the left moved out, as if the internal rectus of the operated eye were insufficiently opposed. After the monkey was well out of the anesthetic it made several attempts to move the eyes straight out to the right; this still resulted in an upward movement of the right

eye. The first movements, therefore, were in the direction one would expect if impulses to muscles were coming out over the usual pathways. After some fifteen minutes the upward movement of the operated eye synchronous with inward movement of the other eye ceased and the eye failed to pass beyond the midline. At this time the monkey attempted to roll its eyes in a complete circle. This was accomplished perfectly with the blind eye, but the operated eye traced a half circle only, for instead of completing the temporal half of its swing, the eye moved straight down the vertical midline. For three days after the operation the right eye was not seen to pass out beyond the midline and a pronounced squint was present. A week later the squint was much less pronounced and movement out was fairly good. Observations were made on this monkey for over a month. The squint still persisted, though it continued to improve and the range of movement continued to increase (fig. 1, G). It must be remembered that in this monkey the unoperated eye was blind, therefore there could be no incentive to straighten the eye in order to overcome diplopia. The improvement in movement was the result of better control of the transplanted muscles. At autopsy it was found that the attachment of the transplanted superior rectus was too slack to hold the eye out in a normal position, though the direction of its pull was very nearly horizontal. The external rectus came obliquely over the back of the eyeball so that the direction of its pull was slightly out as well as up.

This same sequence of events was observed in dogs, viz., the first movements of the operated eye which accompanied inward movement of the unoperated eye were up and out, showing that the transplanted external rectus was still acting in conjunction with the internal rectus of the normal eye; but within half an hour movements of the operated eye almost ceased altogether, and the animal either closed the lid when the normal eye moved up or out, or the eye stopped at the midline in its outward movement. By the following day there was definite outward movement, though the excursion was slight, and in certain cases there was still tendency for the eye to turn up instead of out. In one dog it was observed that when the operated eye failed to assume the proper position the animal would wink and the position would be corrected. This correction after winking was noted in still another dog on the day after the operation for other movements as well as outward movement, particularly the return to the forward position. This behavior suggests that there is gradual adjustment to the new situation. To test whether this readjustment could take place without visual impressions, the eyes of several animals were bandaged while the animals were still under the anesthetic, and observations made on the following day. This experiment was only completely successful in one dog since the others scratched the bandage loose. Immediately on removing the bandage the operated eye was found to turn up when the

normal eye turned in (fig. 1, H). This condition was much improved a week later (fig. 1, I), though there was still a tendency to move the eye up instead of out if the action was a very quick one (fig. 1, J); there was also a distinct lag.

About a month after transposing the muscles, when eye movements were apparently normal, one dog and one cat were deeply anesthetized and the eye area of the opposite cortex cauterized. This had no appreciable effect on the eye movements. There was still, as far as one could observe, perfect coördination. Moving pictures were taken of this cat's eyes and a study of these pictures gave no indication of incoördination. Three months after this first brain operation the eye area of the other cortex was cauterized in the dog. Again no change in eye movements was observed either by direct observation or by inspection of moving pictures taken after this operation. Removal of the eye area of the cortex therefore does not make the animal lose the acquired skill in the use of these transplanted muscles. In other words, the change from the normal nervous pathway to the newly acquired one involves structures more deep-seated than the cortex.

SUMMARY

Evidence is presented that, when certain eye muscles in dogs, cats and monkeys are transposed, there is readjustment so that the transposed muscles can carry out coördinated movements in directions quite different from their original ones. This readjustment does not take place immediately but involves a short period of training. Elimination of the "eye area" of the cortex does not interfere with this readjustment once it is established.

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REFERENCES

DUANE, A. Arch. Ophth. **48:** 1, 1919.
FEARING, F. Reflex action. Baltimore, 1930.
KNUTSSON, F. Acta Oto-Laryng. **13:** 116, 1929.
MARINA, A. Neurol. Centralblatt **34:** 338, 1915.
RUSSELL, J. S. R. J. Physiol. **17:** 378, 1894.

ACOUSTIC VALUE OF THE SEVERAL COMPONENTS OF THE AUDITORY SYSTEM IN CATS[1]

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In a recent study from this laboratory (5) the question was explored: how much does each functional component of the auditory system in dogs contribute to actual hearing? What loss in acuity follows a given structural lesion? The animal's normal threshold at 1000 cycles was first established; some part of its acoustic system was then removed by surgical means and its hearing again measured; a second and finally a third surgical entry eliminated other members of the system from functional participation, whereupon the remanent acuity was in each case again tested. By this means the significance of the following components of the acoustic mechanism was estimated in terms of the dog's residual hearing after they had been functionally excluded: one and both cochleae, one and both cortical hemispheres, crossed and uncrossed fiber-tracts of the lateral lemnisci. These observations, seeming to contravene certain accepted views, were received in some quarters with a measure of skepticism. While they were deemed by us to be sound in both fact and interpretation, we yet recognized that the underlying data were at one point not quite satisfying. The first dog, doubtless because our technical procedures had not then been wholly perfected, failed to agree in its testimony with later animals. It seemed wise, therefore, both to renew and to extend the investigation by using a wider range of test-frequencies, another species of test-animal, and a newly developed apparatus for measuring the sensory performance of small animals.

PROCEDURES. The test-procedures were much like those previously employed. As with dogs, so with cats: once conditioned to a tonal stimulus, the animal continues to respond as the tone is made progressively

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weaker until after a while it begins to falter, reacting sometimes but not always. Upon further reduction of intensity, the cat fails eventually to give any reply to the stimulus; this can only be interpreted to mean that the sound has now become wholly inaudible. Very special care is used to keep the conditioned activity intact, thereby assuring a definite, visible response whenever the cat hears the tone at all. By use of these and other precautions, standardized in this laboratory through several years (cf. 3), the auditory threshold of cats or dogs can be measured with a stability (standard error usually lower than one decibel) fully equal to the performance of graduate students.

In respect of test apparatus, however, we departed from our former procedure of dog-in-stock, trained to respond by retracting the right forepaw from a charged grid. Instead, the cat was placed in a modified "squirrel cage," adapted from the rotary cage commonly used for measuring gross activity in small animals. It is so constructed that the cat, upon turning the cage about one inch when the tone is sounded, opens a pendulum-switch in the high-voltage circuit and thereby escapes the shock (full description in 2). This combination of cat and rotary cage, here used for the first time in a systematic investigation, fully met the exacting conditions of post-operative testing. The reader need hardly be reminded that a cat, just after cortical ablation of one hemisphere, is quite a different test-object than it was just before. This question accordingly must always be faced: will the test-procedures, despite this temporary derangement of the animal's vital economy, give just as reliable measures after as before? The evidence presented below proves, in our opinion, that such was here the case. Indeed, we found the cat-rotary setup equal in reliability and markedly superior in efficiency to the conventional dog-stock combination.

2. Three test frequencies (125, 1000, and 8000 cycles) were used, in order to test for functional differentiation among the lemniscal fibers in transmitting higher and lower frequencies. It is quite conceivable that the relative participation of fibers in the crossed and uncrossed lemnisci may vary with the stimulus-frequency.

3. The operative procedures were much as before and need not be described at length. The peripheral receptors were destroyed by actual fracture of the cochleas. Middle-ear lesions (disarticulation of the ossicles, packing the tympanic cavity with wax) were avoided, their ineffectuality having been demonstrated before (5, p. 481). The vestibular disturbance which follows cochlear destruction soon passed away; in every case the cat was ready for testing the same day.[4] Cortical abla-

[4] The advantage of motivating the test-animal with shock instead of food clearly appears when the cat is still somewhat dizzy and nauseated from cochlear fracture; it can be accurately tested in the rotary-cage at a time when food has no incentive-value whatever.

tion, as might be expected, proved far more serious. In the first two cases, testing was resumed within 12 hours (cat 1) and 48 hours (cat 2) after operation; in the last two cases, however, the animals were not in condition for testing until four or five days had elapsed.

The operative intervention was in every case carefully checked by detailed examination of the gross specimen post mortem and the cortical ablations verified. A summary statement of the situation in each animal, as revealed upon autopsy, follows: *Animal 1*—decortication of one hemisphere complete, no sub-cortical damage. *Animal 2*—decortication of one hemisphere complete, no sub-cortical damage. *Animal 3*—decortication of one hemisphere complete; head of caudate nucleus had been somewhat invaded; basal hemorrhage was found on right side, lateral to septum and anterior to head of caudate nucleus; right rhinencephalon had been somewhat invaded. *Animal 5*—decortication of one hemisphere complete; internal capsule had been somewhat invaded; blood-clot in third ventricle.

In order to verify complete functional elimination of one cochlea, the other cochlea was fractured in the same way and hearing again tested. Inasmuch as the cats then gave no response to tonal stimulation 125 decibels above their normal thresholds (which failure may be accepted as proof of total deafness to air-borne sounds), it is clear that the first cochlea had been functionally destroyed. We wish to constate that the report of a pistol (time-honored method of testing for deafness in laboratory animals) is gravely inadequate. A guinea pig, whose threshold had risen 75 decibels above its normal value after cochlear injury and which therefore had about 50 or 60 decibels of remanent hearing, gave no perceptible startle-reflex of any kind when a pistol (0.32 caliber) was discharged on several occasions a few feet away. Since guinea pigs are commonly assumed to display particularly obvious reflex-indications of hearing, we see how little the pistol-test means. The reason is obvious: to an animal with hearing thus impaired a pistol-shot sounds no louder than, say, ordinary conversation to a normal animal; why then should we expect it to be "startled?" This reveals the superiority of the conditioned method of testing for sensory acuity, where the animal is *trained* to respond to the *faintest* audible sounds. In the above case, the residual acuity was clearly demonstrated by the conditioned technique.

EXPERIMENTAL OBSERVATIONS. 1. *Acoustic value of the cochlea.* In animals otherwise intact, the temporal bulla of one ear was entered by a ventral approach and the cochlea broken up. The hearing-losses range (table 1) from 1 to 5 decibels on the three frequencies (cats 1, 2, 3, 4). Inasmuch as the losses are not consistent either for a given animal or for a given frequency, it follows that the variations are due to errors in sampling. Table 2 gives the mean loss at each frequency for the four animals

(4.1 db at 125 ~, 3.1 db at 1000 ~, 3.0 db at 8000 ~). In the former study, hearing dropped about 3 db with one cochlea out (5, p. 479). This relatively small impairment agrees in order of magnitude with that which attends hemidecortication. This fact seems logical since each cochlea serves one crossed and one uncrossed set of lemniscal fibers; likewise the

TABLE 1*

CAT	FREQUENCY	NORMAL LIMEN	LOSS IN ACUITY FOLLOWING			
			Destruction of		Ablation of	
			Left cochlea	Right cochlea	Left cortex	Right cortex
1	125	*0.0* (0.75 5)	*5.1* (0.90 4)		*22.6* (0.87 6)	
	1000	*0.0* (0.49 5)	*3.2* (0.99 4)		*24.2* (0.99 6)	
	8000	*0.0* (0.80 5)	*1.2* (1.18 4)		*12.9* (1.45 6)	
2	125	*0.0* (0.40 5)		*1.6* (1.10 5)	*14.4* (0.65 6)	
	1000	*0.0* (1.02 5)		*1.2* (1.20 5)	*16.9* (1.08 6)	
	8000	*0.0* (0.75 5)		*4.4* (1.47 5)	*21.7* (0.85 6)	
3	125	*0.0* (0.63 5)	*4.4* (0.98 5)			*14.4* (1.33 5)
	1000	*0.0* (0.63 5)	*4.0* (0.89 5)			*17.2* (1.20 5)
	8000	*0.0* (0.75 5)	*3.2* (0.89 5)			*16.0* (0.85 5)
4	125	*0.0* (0.80 5)		*5.2* (1.02 5)		
	1000	*0.0* (0.40 5)		*4.0* (0.85 5)		†
	8000	*0.0* (1.02 5)		*3.1* (1.95 6)		
5‡	125	*0.0* (0.62 6)		*14.3* (0.62 2)		*2.3* (0.62 2)
	1000	*0.0* (0.34 6)		*17.3* (1.05 2)		*5.3* (1.05 2)
	8000	*0.0* (0.67 6)		*20.3* (1.20 2)		*3.3* (0.67 2)

* Interpretation: The normal limen is always set at 0.0. This does not mean that the five cats start with identical thresholds, but simply that all changes are measured from the animal's normal level. The loss in hearing after a given operation is therefore directly shown by its distance from zero. The first number in each parenthesis is the standard error of the mean directly preceding the parenthesis; and the second number in the parenthesis is the number of independent measures from which the mean is derived and by which the standard error is computed. The means are graphically reproduced in the bar diagram of figure 1.

† No tests—animal succumbs to operation.

‡ Order of procedure reversed; right cortex ablated first, then right cochlea destroyed. In all other cases, cochlea was destroyed first, followed by cortical ablation.

cortex of each hemisphere receives the impulses delivered by one crossed and one uncrossed lemniscal tract.

2. *Acoustic value of the lateral lemnisci.* Auditory impulses from the cochlea reach the brain via the lateral lemnisci. Since each of the auditory nerves contributes to both lemnisci, these tracts contain crossed as

well as uncrossed fibers. After one-half of the cortex has been ablated, the only cortical terminations still intact belong to the hetero-lateral lemniscus. We can thus measure the relative contribution of crossed and uncrossed fibers by removing one or other cochlea. With the right cortex already out, destruction of the *right* cochlea will obviously eliminate from functional participation the crossed (hetero-lateral) component of the left lemniscus. Destruction of the *left* cochlea, on the contrary, will eliminate the uncrossed (homolateral) fibers of the left lemniscus. Table 1 and

TABLE 2

Losses in hearing following ablation, or functional exclusion, of the various components of the acoustic system

	TEST FREQUENCY		
	125 cycles	1000 cycles	8000 cycles
One cochlea (Cats 1, 2, 3, 4)	Mean 4.10 (0.84)	Mean 3.10 (0.44)	Mean 3.00 (0.44)
One cortex (Cat 5)	*2.30*	*5.30*	*3.30*
Uncrossed tracts (Cats 2, 3)	12.80	15.70	17.30
	10.00	13.20	12.80
	Mean 11.40	Mean 14.45	Mean 15.05
Crossed tracts (Cats 1, 5)	17.50	21.00	11.70
	12.00	12.00	17.00
	Mean 14.75	Mean 16.50	Mean 14.35
Left cortex (Cats 1, 2)	17.50	21.00	11.70
	12.80	15.70	17.30
	Mean 15.15	Mean 18.35	Mean 14.50
Right cortex (Cats 3, 5)	10.00	13.20	12.80
	12.00	12.00	17.00
	Mean 11.00	12.60	14.90

figure 1 exhibit in compendious form the losses due to the four possible combinations:

Animal 1, left cochlea—left cortex
Animal 2, right cochlea—left cortex
Animal 3, left cochlea—right cortex
Animal 5, right cochlea—right cortex

In table 2 we find the mean loss at each frequency for both crossed and uncrossed tracts. Do the crossed fibers, which are far more numerous than the uncrossed, have a functional value correlative with their numbers? Let us consider the figures for the two groups respectively. At 125 cycles the crossed fibers surpass in functional significance the un-

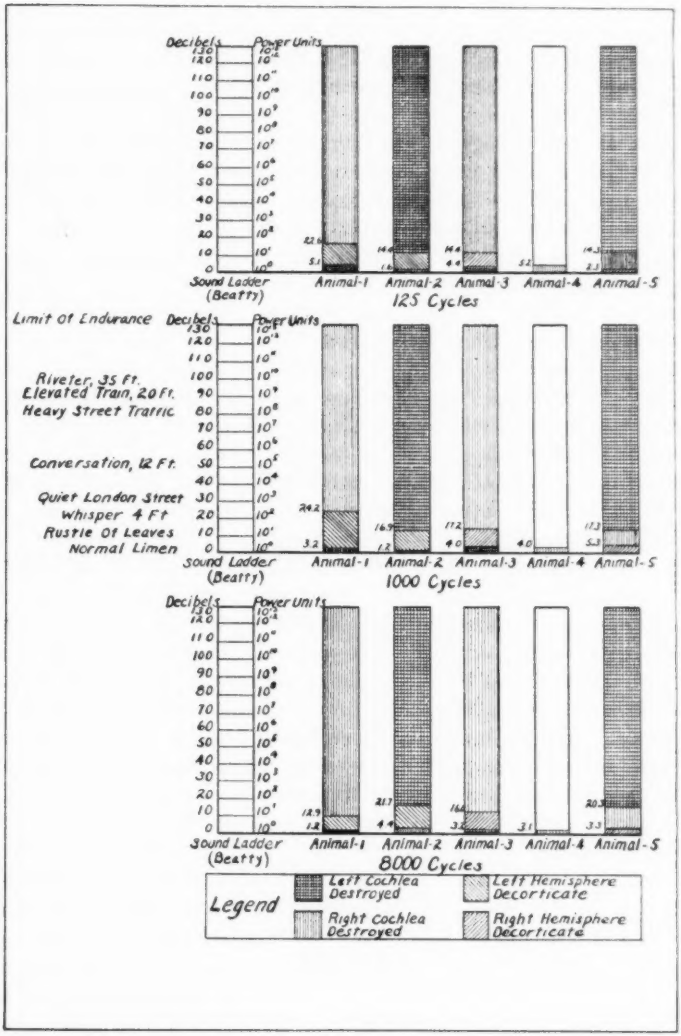

Fig. 1. Diagram showing losses in hearing after ablation or functional exclusion of the various components of the acoustic system, as indicated. On the left the approximate hearing-range is represented as 13 bels or 130 decibels from the faintest audible sound to the "limit of endurance." The "sound-ladder" [adapted from Beatty (1)] interprets these intensity-levels in terms of familiar sounds. The physical power-units rise from unity (10°), which is here set at liminal intensity for a normal ear, to the gigantic figure of 10^{13}. Further explanation in text.

crossed by 3.35 db. (s.e. = 1.29), at 1000 cycles by 2.05 db. (s.e. = 1.50), and at 8000 cycles by −0.70 (s.e. = 1.57). Combining these figures the mean difference in favor of the crossed fibers is 1.57 (s.e. = 0.84). When a difference is three times as large as its own standard error, it is conventionally treated as "real" (not due to sampling errors). We find, however, no pair of figures here whose difference meets this criterion; we may therefore assume, without more ado, that the differences which appear are referable to random sampling, and that the crossed and uncrossed components are functionally equal. From these figures, then, several inferences are justified: *a*. There is no substantial differentiation among the lemniscal fibers in transmitting higher and lower frequencies; they seem to participate about equally at all frequency-levels. While the crossed fibers do seem to contribute more than the uncrossed to the animal's hearing at 125 cycles, their apparent disparity is not reliably greater than zero. *b*. For all frequencies combined, the uncrossed tracts of the auditory system are virtually equal in acoustic significance to the crossed fibers; statistically they belong to the same population. *c*. The mean impairment from loss of one cochlea being 3 to 4 decibels, and the added impairment from loss of one (crossed or uncrossed) lemniscal tract being 13 to 15 decibels, we see that the animal suffers a functional loss of only 16 to 19 decibels even when but *one* of the four main highways to the upper brain-centers is still open. This fact suggests the existence in the acoustic system of a liberal margin or *factor of safety*.

3. *Acoustic value of the cortex*. In only one case (cat 5) was one-half of the cortex ablated prior to destruction of either cochlea; the hearing-losses were 2.3 db., 5.3 db., 3.3 db., a mean value of 3.6 db. (cf. table 2). As previously noted, these figures are indistinguishable from the figures for the loss of one cochlea. In both cases the impairment seems surprisingly small; so small indeed as to be detectable only with accurate methods of measurement. But may not the left half of the cortex be more important for hearing than is the right half? Consider the figures for the left and right cortex, respectively. At 125 cycles the left hemisphere exceeds the right in functional value by 4.15 db. (s.e. = 1.29), at 1000 cycles by 5.75 db. (s.e. = 1.50), and at 8000 cycles by −0.40 db. (s.e. = 1.57). Combining these figures the mean difference in favor of the left hemisphere is 3.17 db. (s.e. = 0.84). Using the conventional criterion, we could say that the left cortex excels the right in acoustic value at 125 and 1000 cycles, and also for the combined figures. The presumption that we are here dealing with a functional superiority of the left cortex (small indeed but genuine) cannot be ignored. Let us examine the material further. Each measure of a given cat's limen is but one sample from a whole population or distribution of limens, all of which are contaminated with variable errors due to casual changes both in the animal itself and in the

stimulating situation. The two means with which we are here concerned incorporate together 51 original measures; left cortex 30 limens, right cortex 21 limens. If we think of these 51 limens as drawings from the same urn (a population whose composition is known to be constant) and combine any 30 of them into one mean (representing left cortex) and the remaining 21 into another mean (representing right cortex), what is the likelihood of our getting two means whose difference would be as much as 3.77 (3.17 ÷ 0.84) times its own standard error? The chances are less than 1 in 1000 (cf. 4). This means that, for cats of the *same kind* as those tested by us, the left cortex is almost certainly superior in acoustic value to the right, at least for the low and medial frequencies; the two belong, in other words, to urns of *unlike* composition. But is this apparently genuine disparity referable to other causes than left and right cortex? While the two *right* ablations were technically not quite perfect, being attended with minor invasions of the caudate nucleus or internal capsule, this subcortical damage would presumably have no effect upon our hearing-tests; but would in any event assuredly not *improve* the animal's hearing. This contingency may therefore be dismissed. A second possibility is that the animals with right ablations, not being tested until 3 or 4 days later than the animals with left ablations, owing to delay in post-operative recovery, might have improved during that period. On this point we have definite evidence. The final hearing-tests were made, in cat 1 six days after, in cat 2 eight days after, left hemi-decortication had been effected. In not one case were the final limens, for all frequencies in both cats, improved over the *average* for the entire six post-operative tests; or to put it otherwise, the final post-operative test, though later by five and six days respectively than the first test, was in three cases identical with it; in two cases was superior by 2 and 4 decibels, and in one case was inferior by 2 decibels. Changes in threshold during the first six or eight days after operation may therefore be dismissed as negligible.

In view of all the evidence we can say with considerable, but not complete, assurance that the two halves of the cortex in cats are not exactly equal in acoustic function; any disparity which may exist favors the left hemisphere but is at most inconsiderable.

Discussion. In addition to the specific results above set forth, one or two comments may be added. As in the former study, the homolateral fibers in the cat prove to be just as significant for purposes of hearing as do the anatomically much more extensive crossed tracts. Likewise the superiority of the left cortex if genuine is relatively inconsiderable. While dominance of the left hemisphere may well be more pronounced in man than in cat or dog, these results suggest that marked disparity in sensory function does not generally characterize the two

hemispheres in higher animals. Most of all these figures reveal a safety factor in the acoustic system such that serious anatomical lesions have but little functional effect. A loss of 50 per cent of the organism's acoustic equipment (by unilateral cochlear destruction or by ablation of one half of the cortex) reduces the animal's sensitivity-range by only 2 or 3 per cent. It seems unlikely that a loss of 3 decibels in acuity would appreciably impair the animal's chances for survival; in which case we may properly speak of a safety factor of two. Indeed we may question whether the mortality-ratio among cats would be seriously increased by a loss of three-fourths of their normal acoustic equipment, that is, by a functional attenuation in hearing of 16 to 18 decibels (safety-factor of four). In any event nature seems to have used a safety-ratio which would meet the requirements of modern engineering standards.

It is this safety-factor which serves to explain why the crossed and uncrossed fibers, though anatomically unequal in number, are functionally equivalent. The oft-cited analogy of the "burning fuse" may serve to picture the situation. One might say that each cochlea employs two paths, one of a single fuse (uncrossed fibers) and one of two or three fuses (crossed fibers). The single fuse will be "activated" with just as low an input in the cochlea and will in turn "activate" the higher centers just (or almost) as effectively as will several fuses. The main advantage of having more than one "fuse" is that, when one is damaged, the others may carry on.

SUMMARY

1. Destruction of one cochlea, in a cat otherwise intact, is followed by a hearing loss of 3 to 4 decibels. Destruction of both cochleae eventuates in total deafness to air-borne sounds (no response to tones 125 decibels above the animal's normal threshold).

2. Ablation of a single half of the cortex is followed by a hearing loss of the same magnitude—3 to 5 decibels. Statistical analysis indicates that the left hemisphere may be slightly superior in acoustic value to the right; but in any event there is no substantial disparity.

3. In cats with one half of the cortex ablated, destruction of the heterolateral cochlea causes an additional drop in acuity, owing to exclusion of the *crossed* fibers of the lateral lemniscus, of 15 decibels.

4. In cats with one half of the cortex ablated, destruction of the homolateral cochlea causes an additional drop in acuity, owing to exclusion of the *uncrossed* fibers of the lateral lemniscus, of 13 to 14 decibels.

5. It follows that the uncrossed fibers of the auditory system are virtually equal in acoustic significance to the crossed components. Statistical analysis fails to reveal even a presumptive superiority of the crossed pathways.

6. The evidence discloses a satisfactory safety-factor in the acoustic system, which compares favorably with that found in other physiological systems and with good engineering practise.

7. By use of testing-equipment recently developed in this laboratory, it was made feasible to institute a systematic program of quantitative hearing-tests in post-operative cats.

8. The above conclusions confirm and considerably extend conclusions previously derived in this laboratory from dogs.

REFERENCES

(1) Beatty, R. T. Hearing in man and animals. London, 1932.
(2) Brogden, W. J. and E. Culler. Science **83:** 269, 1936.
(3) Culler, E., G. Finch, E. Girden and W. Brogden. J. Gen. Psychol. **12:** 223, 1935.
(4) Fisher, R. A. Metron **5:** 90, 1925.
(5) Mettler, F. A., G. Finch, E. Girden and E. Culler. Brain **57:** 475, 1934.

THE INFLUENCE OF THE PLANE OF NUTRITION ON THE MANNER OF HEAT DISPOSAL BY CATTLE[1]

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The question of the manner of disposition of heat by the animal body is of especial interest and importance, because it is intimately connected with the relation between the insensible loss in weight and the energy metabolism, and with the problem of determining the heat production without the aid of a calorimeter or respiration apparatus. The heat produced in the body is eliminated in two principal ways: 1, by radiation and conduction, and 2, by vaporization of water from the skin and lungs. A relatively small amount of heat is lost through warming the inspired air and the ingested food and water. Because heat elimination practically always equals heat production (in homotherms), an inverse relation exists between the proportions of heat production which are liberated through the two principal channels. Any factor, therefore, which influences in a certain way the proportion of heat eliminated through vaporization of water will have the opposite effect on the proportion of heat dissipated by radiation and conduction, and vice versa.

Although various factors affecting the vaporization of water have been investigated, relatively little attention has been given to the influence of the amount of food consumed on the vapor heat quotient, that is, on the percentage of heat production represented by the latent heat of water vaporized.

It is a well established fact that food consumption causes an increase in the amount of water vaporized and in the total insensible weight loss. Evidence to this effect is found in the reports of Benedict and Ritzman (7), Kriss (21) and Hovland (19). Is the increase in water vaporization a direct function of the increase in metabolism? The answer to this question depends on whether or not the vapor heat quotient is the same under feeding conditions as under conditions of fasting, and on whether or not this quotient remains constant under different planes of food intake. These questions are of importance, especially, as applying to subjects undergoing marked changes in the plane of nutrition, such as

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frequently occur in nutrition investigations with animals. Definite information on these points is lacking.

In a statistical study of respiration calorimetric data for cattle Kriss (22) has found a considerably higher coefficient of correlation between the dry matter of the food consumed and the heat production than between either the water vapor or insensible weight loss and the heat production. These findings suggested a possible influence of the amount of food consumed on the proportion of heat lost through vaporization of water.

In order to obtain more definite evidence regarding the way the disposal of heat is influenced by the plane of nutrition, a further analysis has been made of the respiration calorimetric data of the Pennsylvania Institute of Animal Nutrition, approaching the problem from a different angle. This constitutes the basis of the present paper.

In this study changes in the vapor heat quotients are compared directly with changes in 1, the metabolizable energy of the rations; 2, water consumption, and in 3, the total heat production of the same experimental animals. Food consumption appears to have such a definite influence on water consumption that any study of the influence of plane of nutrition on the water vaporization must also include a consideration of the amount of water consumed.

DATA. The data upon which the present paper is based were derived from twenty series of energy metabolism experiments with steers and six series of such experiments with cows, all of which were conducted with a respiration calorimeter. The experiments with steers were conducted with sixteen individual animals and comprise ninety-nine experimental periods. The experiments with cows comprise twenty-one experimental periods involving four individual animals.

Each of these series of experiments, except two, includes from three to seven experiments which were conducted during one season with one and the same animal on different planes of food intake. In some of these series the planes of nutrition varied from fasting to full feed.

Several of the published calorimetric experiments were omitted from the present study, either because they did not involve any considerable variations in the planes of nutrition, or on account of some other experimental conditions which rendered them unsuitable for the present purpose.

The data include 1, the metabolizable energy of the daily ration; 2, the daily water consumption; 3, the daily heat production, and 4, the latent heat of water vaporization as per cent of the total heat production.

The data for heat production used in this study are those obtained by direct calorimetry during periods of two or three days. The amounts of water consumed and of water vaporized were determined for the same intervals. The temperatures of the calorimeter were very uniform, especially during any one series of experiments here considered.

TABLE 1

The percentages of heat eliminated by vaporization of water by cattle at different planes of nutrition

EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS	EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS	EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS
Steers		*per cent*	Steers		*per cent*	Steers		*per cent*
200-A (1)	1	22.4	212-H (10)	1	24.3	240-57 (14)	2	26.6
	2	26.7		2	24.5		4	32.4
	3	19.4		3	22.6		6	43.8
	4	22.5		4	22.9		8	43.9
				5	20.2		10	27.7
207-A (1)	1	21.7		6	20.7		12	28.7
	2	29.5					14	28.1
	3	21.9	216-J (5)	1	37.9			
	4	24.7		2	27.0	261-B (16)	2	33.1
				3	28.4		4	37.4
200-B (1)	1	25.2		4	23.2		6	35.6
	2	23.7		5	32.0		8	27.0
	3	20.4		6	29.6		10	32.3
	4	21.1		7	24.2		12	31.3
							14	36.1
207-B (1)	1	22.7	217-J (4)	1	24.2			
	2	31.8		2	48.1	Cows		
	3	26.1		3	45.9	221D-885 (8)	1	34.1
	4	29.1		4	32.1		2	40.0
							3	34.2
210-D (2)	1	24.0	220-K (6)	1	24.0		4	25.8
	2	23.2		2	21.9			
	3	19.7		3	23.3			
				4	28.6	221E-885 (8)	1	39.7
211-D (3)	1	23.5		5	28.9		2	32.8
	2	22.7					3	22.3
	3	26.9	235-259 (12)	2	20.4			
	4	20.6		3	24.4	221D-886 (8)	1	30.7
	5	21.0					2	38.8
			235-260 (12)	2	35.3		3	27.6
208-E (2)	1	23.3		3	37.7		4	20.7
	2	20.8						
	3	20.6	238-47 (13)	3	32.7	221F-874 (8)	1	31.3
	4	25.3		5	26.0		2	26.1
	5	22.5		7	27.3		3	22.0
	6	21.3		9	33.2			
				11	27.6	221F-887 (8)	1	30.2
209-F (2)	1	23.5					2	22.8
	2	19.8	238-36 (13)	2	20.4		3	19.2
	3	22.0		4	32.4			
	4	31.1		6	24.0	221G-887 (17)	1	28.3
	5	18.2		8	26.8		2	21.4
	6	22.8		10	29.5		3	25.0
				12	24.1		4	31.9

TABLE 1—*Concluded*

EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS	EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS	EXPERIMENT, ANIMAL AND REFERENCE	PERIOD NUMBER	VAPOR HEAT QUOTIENTS
Steers		*per cent*	Steers		*per cent*			*per cent*
211-G (3)	1	24.1	240-60 (14)	1	22.2			
	2	21.8		3	27.6			
	3	34.7		5	38.2			
	4	25.5		7	46.2			
	5	23.1		11	22.4			
				13	28.7			
				15	23.7			

For each period the percentage of heat lost through vaporization of water (the vapor heat quotient) was calculated on the basis of data for water vapor which include the water that was condensed on the cooling system in the calorimeter. This calculation was necessary because, while in all the published balances of water from this Institute the data for water vapor do include the water condensed in the calorimeter, the data for latent heat of water vapor are in some cases based on the quantities of water measured in the air current only. The recalculated vapor heat quotients are given in table 1.

All data (except for expt. 235) are presented graphically in figures 1 to 4. The experimental periods in each series are arranged in the order in which the experiments were originally conducted, and as indicated in table 1. The successive periods are used as abscissae, and the values for metabolizable energy, heat production, water consumption and vapor heat quotients are used as ordinates.

Discussion. The graphs are, in a large measure, self-explanatory. They reveal in a striking manner not only that the vapor heat quotients are influenced by the intensity of the metabolism but that the changes in these quotients follow more closely the changes in the total metabolizable energy of the ration than they do the changes in the heat production. This indicates that while the metabolism is increased with an increase in food consumption, the amount of water vaporized is increased in larger proportions than is the total heat production.

The metabolizable energy curve together with the curve of heat production show at a glance what the plane of nutrition was for the animal in any period. Since the difference between the metabolizable energy of the ration and the heat production equals the energy gain or loss by the body, any point on the metabolizable energy curve lying above the curve of heat production represents supermaintenance feeding; those points lying below the heat production curve represent submaintenance planes, while the point of intersection of the two curves indicates energy equilibrium.

It is important to note, in the graphs, how closely the quantities of water consumed (in kilos) follow the metabolizable energy of the rations (in Therms). While the feeding of the animals was controlled, water was offered to the animals once or twice during twenty-four hours, ad libitum. The animals themselves then regulated their intake of water in accord

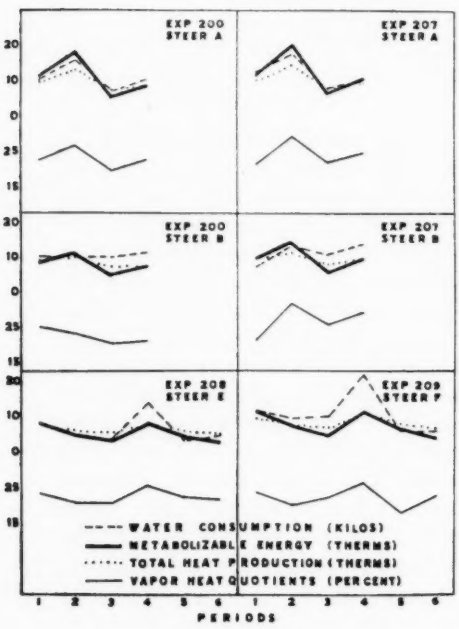

Fig. 1

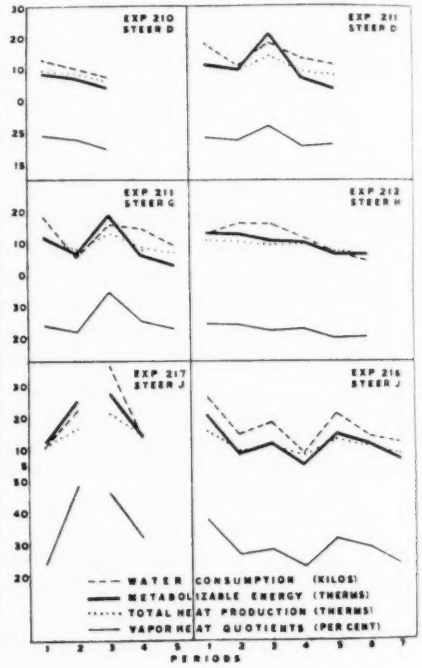

Fig. 2

Fig. 1. The relation between metabolizable energy of the ration, water consumption, heat production and the percentage of heat eliminated through vaporization of water by steers.

Fig. 2. The relation between metabolizable energy of the ration, water consumption, heat production and the percentage of heat eliminated through vaporization of water by steers.

with the ration. It is possible that this adjustment of the water intake to the rations fed is one of the important factors which are immediately responsible for the changes in the vapor heat quotients which accompany the changes in the planes of nutrition. Indeed, the graphs indicate that in most of the experiments the water consumption and the metabolizable energy of the rations are about equally well correlated with the vapor heat quotients.

Whether water administration *per se* influences the rate of vaporization of water from the body, or the insensible weight loss, is a question concerning which there is considerable controversy in the literature. Schwenkenbecher (26), using the chamber method for measuring the water evaporated from the skin directly, reported that the administration of large quantities of water had no effect on the rate of vaporization of water from

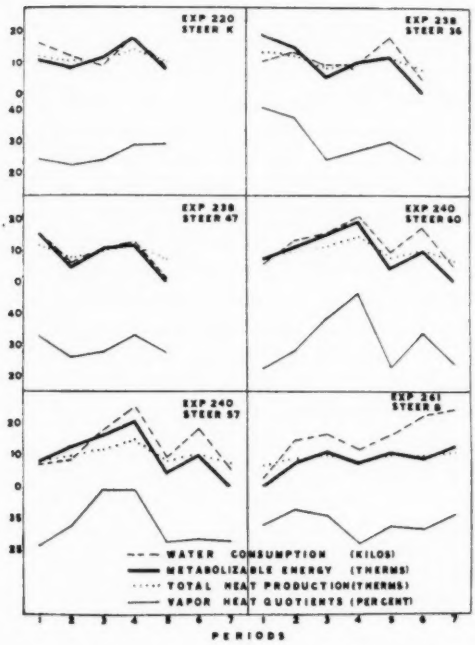

Fig. 3

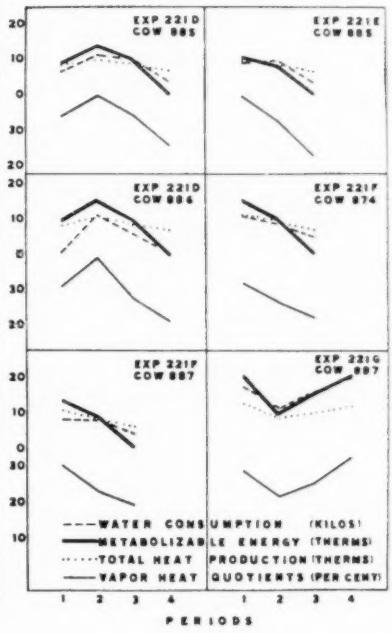

Fig. 4

Fig. 3. The relation between metabolizable energy of the ration, water consumption, heat production and the percentage of heat eliminated through vaporization of water by steers.

Fig. 4. The relation between metabolizable energy of the ration, water consumption, heat production and the percentage of heat eliminated through vaporization of water by cows.

the skin. Erisman (9), Moog and Mauck (24), and Manchester, Husted and McQuarrie (23), however, observed a relationship between the water consumption and the insensible loss. The latter investigators also found that the type of diet and the mineral content of the ingesta apparently influence the insensible loss, and suggest that these factors should be considered in the interpretation of results when the regimen is undergoing sudden changes or is extreme in any respect.

Newburgh and Johnston (26) made the observation that the discomfort produced by the curtailment of water led their subject (a man) to omit many of his accustomed activities, with the result that the insensible loss was lowered. In a subsequent experiment, with the same subject, they found that dehydration to the extent of removing approximately 6 per cent of the body water, was without effect on the percentage of heat loss by vaporization of water.

Gilman and Barbour (18) measured the rate of insensible weight loss in rabbits before and after administration of hypertonic saline and water in comparison with control subjects receiving isotonic saline. The extent of hydration or dehydration was indicated by measurements of the osmotic pressure of the blood. The authors observed that an increase in blood osmotic pressure caused a marked decrease in the rate of insensible weight loss, whereas a decreased osmotic pressure of the blood resulted in an increased rate of water vaporization from the skin and lungs.

From a consideration of the foregoing it appears that the water consumption can not be overlooked in the interpretation of the parallelism which is shown in our graphs between the amounts of food metabolized and the vapor heat quotients. On the other hand, the water intake can not be regarded as the primary cause for this parallelism, because, while the relation between food and water consumption is probably not fortuitous, the quantities of food metabolized were, of course, not determined by the water intake, the reverse being apparently true.

Since in the normal nutrition of animals it is not practicable to eliminate variations in the water intake, especially when considerable changes in the food intake are made, it would seem impossible, under such conditions, to evaluate the effect of the ration on the manner of heat disposal apart from the accompanying effect of the water consumed. From this point of view it seems necessary to regard the influence of the plane of nutrition on the vapor heat quotients as being represented by the aggregate effect of the food and water consumed.

Another important factor which it is necessary to consider in the interpretation of the results presented in this paper is the coat of hair of the animals. It was shown elsewhere by Kriss (21) that the insensible weight loss of steers was greatly influenced by this factor, and that this influence could not be related to the metabolism.

In a comparison of the insensible loss of steers 259 and 260, experiment 235, it was found that under identical feeding conditions, the insensible loss of steer 260, which had his full winter coat of hair, was considerably greater than that of steer 259 which had been shorn. This is verified by the vapor heat quotients for these animals as shown in table 1. Whereas in periods 2 and 3 these quotients are 20.4 and 24.4 with steer 259, they are 35.3 and 37.7 with steer 260 in the corresponding periods. In connec-

tion with the present study it is interesting to note that in both the shorn and unshorn animals the vapor heat quotients were influenced by the plane of nutrition.

In all steer experiments presented in table 1, except experiments 238, 240, 261, and experiment 235—steer 260, the animals were clipped once at the beginning of the season, and they were brushed before each calorimetric experiment. In experiments 238, 240, 261 and 235—steer 260, and in all the experiments with cows, the animals were not clipped. The relatively high vapor heat quotients observed in some of the experiments with the unclipped animals as compared with those obtained with the clipped animals at comparable planes of nutrition may be due to an appreciable extent to the coat of hair.

In each individual series, however, any influence of the growth of hair from one period to another may reasonably be expected to be in one direction. In view of the fluctuations of the vapor heat quotients in any series of experiments in accord with variations in the rations, regardless of whether the animal was clipped or not, and, in case it was clipped, regardless of the lapse of time from the time the clipping was done, the influence of the plane of nutrition on the vapor heat quotients appears to be very definite.

A factor which is apparently less important than either the temperature of the environment, the water consumption, or the coat of hair of the animal, but which probably contributed somewhat to the variations in the vapor heat quotients, is the relative humidity of the air in the chamber.

The moisture content of the air in the chamber was undoubtedly subject to variations depending not only on the moisture content of the incoming air, but also on the rate of ventilation, on the amount of water vaporized from the animal body, and on the amount of water condensed on the cooling system. In some of the experiments the rate of ventilation was kept constant in the different experimental periods. In others it was occasionally changed from one period to another when a marked change in the plane of nutrition occurred, a greater rate of ventilation being allowed at the higher planes in consideration of the comfort of the animal. It is obvious that even in those experiments in which the rate of ventilation was constant for all the periods, the relative humidity could be affected by the amount of water vapor produced in the chamber.

In a recent investigation by Forbes, Braman and Kriss (15) of the influence of the rate of ventilation and of the relative humidity of the air on the heat production of a cow, it was observed that, with the ingoing air containing its natural moisture, a marked change in the rate of ventilation, which was accompanied by a marked change in the relative humidity of the air in the chamber, had a relatively slight effect on the vapor heat quotients. The effect was greater when the ingoing air was freed from moisture by freezing.

Thus, in four consecutive days, during which the ration was unaltered, but with changes in the rate of ventilation from one day to the other, the relative humidity of the air in the chamber was 68.4, 87.3, 66.3 and 90.0 per cent, and the vapor heat quotients were 39.8, 40.0, 42.2 and 39.1.

When the ingoing air was freed from moisture by freezing, the relative humidity of the outcoming air in four different days was 54.7, 84.2, 58.9 and 86.8 per cent, and the vapor heat quotients in the corresponding days were 41.1, 38.7, 46.1 and 40.6.

It should be clearly understood that the foregoing tests involved very drastic changes in the rate of ventilation, the rapid rate being about seven times the slow rate. Such extreme variations in the rate of ventilation never occurred in any of the series of experiments presented in table 1.

In the light of the foregoing discussion it may be assumed that the variations in the vapor heat quotients in the several series of experiments under consideration, were to an indeterminable extent, affected by the relative humidity, but that the effects were probably not great.

It will be observed, in table 1, that the variations in the vapor heat quotients, even during any one season with one and the same animal, are of considerable magnitude. The largest variations occur in experiments 240 and 217.

In experiment 240 the planes of nutrition varied from fasting to about three times the maintenance requirement. The vapor heat quotients vary with one animal (steer 57) from 26.6 per cent to 43.9 per cent, and with another animal (steer 60) the extreme variation in the quotient is from 22.2 per cent to 46.2 per cent.

In experiment 217 the animal (steer J) was in an unfattened condition during periods 1 and 2. Between periods 2 and 3 the steer was fattened, and he was used in the fattened condition in periods 3 and 4. In the unfattened condition the vapor heat quotient was changed from 24.2 in period 1 to 48.1 in period 2, when the ration was approximately doubled. In the fattened condition the vapor heat quotient was reduced from 45.9 to 32.1 when the ration was reduced to about one-half. In both the fattened and unfattened condition there was a marked response of the vapor heat quotient to the changes in the plane of feeding.

Of especial interest are the data of experiment 212. In this experiment the changes in the plane of nutrition were not very great, but as shown in the graphs (fig. 2) there is a remarkable parallelism between the vapor heat quotient curve and the curve representing the metabolizable energy of the ration, although no such parallelism is shown in this experiment between the water consumption and the vapor heat quotients.

Of the experiments with the cows here considered (fig. 4) all but one series (221G) were conducted with the animals in the dry condition. In experiment 221G there were marked changes in the rations while the

animal was lactating. No particular effect is noticed of this condition of the animal on the relationship between the vapor heat quotients and the planes of nutrition.

It will be observed that in the experiments with cows the vapor heat quotients in the fasting periods were considerably lower than in the periods of maintenance. A similar situation was not found to prevail in the experiments with steers. In these experiments the vapor heat quotients are practically constant for the animal between the fasting and the maintenance levels. Thus, in experiment 238, steer 47, the vapor heat quotients in the fasting, one-half maintenance and maintenance periods (periods 11, 5 and 7) are 27.6, 26.0 and 27.3, respectively. With steer 36 at similar levels (periods 12, 6 and 8) these quotients are 24.1, 24.0 and 26.8. In experiment 240, steer 60, the quotients, at the three levels indicated above (periods 15, 11 and 1) are 23.7, 22.4 and 22.2, and with steer 57 (periods 14, 10 and 2) the quotients are 28.1, 27.7 and 26.6.

These results with steers are in harmony with the observations by Johnston and Newburgh (20) on human subjects that small quantities of food (below maintenance) do not alter materially the percentage of heat loss through vaporization of water. They are, evidently, in conflict with the results obtained with the cows, when the comparison is made between the fasting and the maintenance levels. In the experiments with the cows, however, there was no submaintenance feeding comparable to the low levels of feeding which occurred in some of the steer experiments. In general, it appears that between fasting and maintenance the effect of the ration on the vapor heat quotients is proportionately less than above maintenance.

In an attempt to give numerical expression to the observed parallelism between the metabolizable energy of the rations and the percentages of heat lost by vaporization of water, the differences in metabolizable energy between consecutive periods were calculated in each series of experiments and compared with the corresponding differences in the vapor heat quotients. By dividing the first set of data into the second set ratios were determined which showed the increases or decreases in percentage of heat lost by vaporization of water per 1 Therm (1000 kilogram-calories) increase or decrease in metabolizable energy. It is a significant fact that out of 84 such determinations 80 ratios were positive and only 4 were negative. In other words, in 95 per cent of the cases a change in the metabolizable energy of the ration in any direction was accompanied by a change in the vapor heat quotient in the same direction.

The ratios, however, showed great variability. The mean of all 84 determinations was found to be 0.92 ± 0.191. The standard deviation was ± 0.823, and the coefficient of variability was 89.5 per cent. The average of all (47) ratios for the clipped animals was found to be $0.69 \pm$

0.066 with a standard deviation of ± 0.667 and a coefficient of variability of 96.7 per cent. The average of all (37) ratios for the animals which were not clipped was 1.21 ± 0.105, the standard deviation being ± 0.960 and the coefficient of variability being 79.3 per cent. The data indicate that clipping of the animals reduces but does not eliminate the effects of the plane of nutrition on the vapor heat quotients.

All the foregoing discussion leads to the obvious conclusion that the plane of nutrition influences the manner of heat disposal, relatively greater proportions of heat being liberated by vaporization of water at the higher planes of food intake. This is probably one of the important reasons why the coefficients of correlation between the insensible weight loss and the heat production, and between the water vaporized and the heat production were found by Kriss (22) to be lower than the coefficient of correlation between the dry matter of food consumed and the heat production.

It follows that the computation of the heat production of cattle from the insensible weight loss at markedly different planes of nutrition, including supermaintenance feeding, can not be accomplished with an accuracy sufficient for research purposes by assuming that the percentage of heat lost by vaporization of water is the same at all levels of feeding.

SUMMARY AND CONCLUSIONS

Comparisons of the metabolizable energy of the rations of steers and cows, in 120 experimental periods, with 1, the percentage of heat lost by vaporization of water; 2, the total heat production, and 3, water consumption, reveal the following facts:

There is a striking parallelism between the percentages of heat lost by vaporization of water, Therms of metabolizable energy and kilograms of water consumed.

Changes in the proportions of heat lost by vaporization of water follow more closely changes in the metabolizable energy than they do the changes in the heat production, thus indicating that while the metabolism is increased with an increase in food consumption, the amount of water vaporized is increased in larger proportions than is the total heat production.

In 95 per cent of the comparisons a change in the metabolizable energy of the ration in any direction was accompanied by a change in the vapor heat percentage in the same direction. Hence, greater proportions of heat are lost by vaporization of water at the higher planes of nutrition.

The extreme variation in the percentage of heat liberated by vaporization of water was, with one animal, from 22.2 per cent to 46.2 per cent.

The data indicate that between fasting and maintenance the effect of the ration on the vaporization of water is proportionately less than above maintenance.

Clipping of the animals reduces but does not eliminate the effects of the plane of nutrition on the manner of heat disposal.

The data lead to the conclusion that it would not be justified to assume that individual animals eliminate the same proportions of heat by vaporization of water regardless of the level of feeding.

REFERENCES

(1) ARMSBY, H. P. AND J. A. FRIES. U. S. Dept. Agr., Bur. An. Indus. Bul. **128**, 245 p., 1911.
(2) ARMSBY, H. P. AND J. A. FRIES. J. Agr. Research **3:** 435, 1915.
(3) ARMSBY, H. P. AND J. A. FRIES. J. Agr. Research **10:** 599, 1917.
(4) ARMSBY, H. P. AND J. A. FRIES. J. Agr. Research **11:** 451, 1917.
(5) ARMSBY, H. P. AND J. A. FRIES. J. Agr. Research **15:** 269, 1918.
(6) ARMSBY, H. P., J. A. FRIES AND W. W. BRAMAN. J. Agr. Research **7:** 379, 1916.
(7) BENEDICT, F. G. AND E. G. RITZMAN. Carnegie Inst. Wash. Pub. **377:** 66, 1927.
(8) COCHRANE, D. C., J. A. FRIES AND W. W. BRAMAN. J. Agr. Research **31:** 1055, 1925.
(9) ERISMAN, F. Ztschr. f. Biol. **11:** 1, 1875.
(10) FORBES, E. B., J. A. FRIES AND W. W. BRAMAN. J. Agr. Research **31:** 987, 1925.
(11) FORBES, E. B., J. A. FRIES, W. W. BRAMAN AND M. KRISS. J. Agr. Research **33:** 591, 1926.
(12) FORBES, E. B., W. W. BRAMAN AND M. KRISS. J. Agr. Research **33:** 579, 1926.
(13) FORBES, E. B., W. W. BRAMAN AND M. KRISS. J. Agr. Research **37:** 253, 1928.
(14) FORBES, E. B., W. W. BRAMAN AND M. KRISS. J. Agr. Research **40:** 37, 1930.
(15) FORBES, E. B., W. W. BRAMAN AND M. KRISS. J. Nutrition **5:** 387, 1932.
(16) FORBES, E. B., W. W. BRAMAN, M. KRISS AND R. W. SWIFT. J. Agr. Research **46:** 753, 1933.
(17) FRIES, J. A., W. W. BRAMAN AND D. C. COCHRANE. U. S. Dept. Agr. Bul. **1281,** 36 p., 1924.
(18) GILMAN, A. AND H. G. BARBOUR. This Journal **104:** 392, 1933.
(19) HOVLAND, C. J. This Journal **3:** 448, 1935.
(20) JOHNSTON, M. W. AND L. H. NEWBURGH. J. Clin. Invest. **8:** 147, 1930.
(21) KRISS, M. J. Agr. Research **40:** 271, 1930.
(22) KRISS, M. J. Agr. Research **40:** 283, 1930.
(23) MANCHESTER, R. C., C. HUSTED AND I. MCQUARRIE. J. Nutrition **4:** 39, 1932.
(24) MOOG, O. AND E. F. NAUCK. Ztschr. f. ges. exper. Med. **25:** 385, 1921.
(25) NEWBURG, L. H. AND M. W. JOHNSTON. J. Nutrition **7:** 107, 1934.
(26) SCHWENKENBECHER, A. Deutsch. Arch. f. Klin. Med. **79:** 29, 1904.

ON CARBOHYDRATE METABOLISM IN ADRENALECTOMIZED ANIMALS

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The idea that the adrenal cortex may participate in the regulation of carbohydrate metabolism has formed the basis for one theory (1) explaining the vital importance of this tissue. Opposed to the carbohydrate theory of the nature and cause of adrenal cortical insufficiency is the hypothesis that the adrenal cortex is primarily concerned with the regulation of the metabolism of inorganic salts and water (2, 3).

One phase of endogenous carbohydrate metabolism which is of vital importance to the animal has received little consideration in pathological conditions, namely, the synthesis of liver glycogen from muscle (dextro) lactic acid. The observation that lactic acid is produced only slowly by the autolyzing gastrocnemius muscle of adrenalectomized cats, although blood lactic acid is known to be high in such animals, led to the hypothesis proposed in 1932 by Buell, Strauss and Andrus (4) that glycogenesis from d. lactic acid may not take place readily in the livers of adrenalectomized animals. The present paper reports a systematic study of the fate of d. lactic acid in the tissues of adrenalectomized animals (rats) in a variety of carefully controlled conditions.

EXPERIMENTAL. *Plan of experiments.* The experimental plan adopted was similar to that used previously in the study of a related problem (5). In brief, known quantities of d. lactic acid, brought to pH 7 with sodium hydroxide, were introduced by stomach tube into young male rats which had been fasted for 24 hours. A comparison of the distribution of compounds participating in the metabolism of carbohydrate in control and lactate-fed animals three hours after feeding revealed in part the fate of the ingested lactate.

The power of the liver to form glycogen from absorbed d. lactate was studied in the following conditions: 1, in normal animals; 2, in adrenalectomized animals maintained in chronic adrenal insufficiency by the use of inadequate amounts of extract prepared by the method of Grollman and Firor (6); 3, in adrenalectomized animals maintained in good condition by the substitution of the salt solution of Rubin and Krick (7) for drinking

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water; 4, in adrenalectomized animals maintained in good condition by mixing with the food a potent charcoal adsorbate[2] prepared from beef adrenal glands by the method of Grollman, Firor and Grollman (8), and 5, in adrenalectomized animals sustained by the combined use of inorganic salts and charcoal adsorbate. It was hoped to find out whether or not the critical carbohydrate levels found in adrenal insufficiency were present also in adrenalectomized animals maintained by the use of inorganic salts.

Choice of animal. The rat was chosen as the experimental animal because it is small and because its metabolism is known to parallel that of man in many important respects. Some hesitancy was felt in the use of the rat because of frequent reports of the development of accessory cortical tissue in this species, as reflected by the long survival of adrenalectomized animals without supplementary therapy. Under our conditions, i.e., with the strains of rats, diet, and operative technique used, and a maximum interval of 20 days between operation and sacrifice of the animals, this proved not to be a real danger.[3] This point was frequently and carefully tested. Either one of a group of adrenalectomized rats was selected at random, given no protective therapy, and allowed to die of acute adrenal insufficiency as a test control; or whole groups which were growing rapidly were temporarily deprived of therapy until an adverse effect on the animals had been established as determined by their growth. Daily weight records of the animals were kept. The growth of all the animals compared favorably with that of normal animals which had undergone a sham operation, with the exception of the animals which were allowed to go into insufficiency. These animals lost weight.

Only male rats were used in order to eliminate the effect of the sexual cycle in the female, and to avoid the differences between the sexes in the distribution of carbohydrate in the tissues (9, 10). They weighed between 100 and 150 grams, at the time they were used. The rat offers a disadvantage in the study of this problem, inasmuch as the muscle glycogen in this species falls less abruptly after adrenalectomy than that of the cat (4) and dog (11).

Operative treatment. The animals were kept for a few days on the standard laboratory diet[4] before adrenalectomy. The operation was per-

[2] The charcoal was mixed with the food in amounts which provided each animal about 0.2 gram charcoal daily.

[3] Rats weighing about 100 grams which were adrenalectomized and given either Rubin-Krick (7) salt solution or 0.9 per cent sodium chloride solution, all remained in excellent condition and grew normally during the five months following operation. When at this time tap water was substituted for salt water, most of them died within 7 days, with no evidence of accessory cortical tissue demonstrable pathologically.

[4] The diet had the following composition, expressed in grams per hundred: yellow maize 57, linseed oil meal 12, casein 3.8, alfalfa 1.5, iodized salt 0.4, calcium chloride 0.4, powdered whole milk 25.

formed by the lumbar route under the initial guidance of Dr. W. M. Firor, with phanodorn or nembutal anesthesia administered intraperitoneally. Removal of both adrenal glands took less than five minutes. The animals were kept for a period of at least two weeks before use in order that they might recover from the operative shock and that there might be adequate time for the attainment of the physiological adjustments desired.

Experimental procedure. The lactic acid was prepared by the continuous ether extraction of an acidified solution of the zinc salt of d. lactic acid which was obtained from Prof. W. H. Peterson of the University of Wisconsin ($[a]_D^{20} = -8.25$). It was brought to pH 7 with sodium hydroxide. The amounts of lactic acid given to the animals were varied in such a way that the amounts absorbed by all groups were comparable.

All animals received no food for 24 hours before the experiment. The adrenalectomized animals which were maintained in good condition received suitable protective therapy (charcoal adsorbate or salt water) during the fast. At the beginning of the experiment a stomach tube was passed and 2 ml. of fluid were given,—lactic acid solution to the experimental animals and the fluid which the animal had been drinking (water or salt solution) to the control animals.

After a three hour interval in an individual metabolism cage the animal was given sodium amytal intraperitoneally. The following procedure was carefully timed and accurately controlled in order that the effect of manipulation on both control and experimental animals should be the same. When anesthesia was complete, both gastrocnemii were rapidly dissected out for the determination of glycogen and moisture. The head was cut off, and blood was collected from the neck veins. The entire liver was rapidly excised, part being used for a glycogen determination and part for a moisture determination. The alimentary tract was then tied at each end, dissected out, minced and extracted three times with boiling water for a lactic acid determination. The urine remaining in the bladder was expressed and added to that in the cage for a lactic acid determination.

The chemical methods employed were those previously described (5). The moisture was determined as the loss in weight of the tissue after being heated at 105° for 48 hours. Absorption of lactic acid was calculated as the difference between the amount given and the amount found in the alimentary tract, allowance being made for the small quantities found in the alimentary tracts of the control animals. No significant quantities of lactic acid were found in the urines.

RESULTS AND DISCUSSION. A summary of the results is given in table 1.

Absorption of lactic acid. It was found that in adrenal insufficiency the rate of absorption of lactic acid was greatly reduced as compared with normal animals (62 mgm. as opposed to 108 mgm., in three hours when 170 mgm. were given per 100 grams body weight). Cori and Cori (12)

TABLE 1

Summary of results

OPERATIVE TREATMENT	SPECIFIC THERAPY	CONDITION	GROUP		NUMBER OF RATS	LACTIC ACID			LIVER		MUSCLE		BLOOD	
						Given	Absorbed		Water	Glycogen	Water	Glycogen	Sugar	Lactic acid
						mgm. per 100 gm.	*mgm. per 100 gm.*	*per cent of total*	*gm. per cent*	*mgm. per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
None	None	Normal	1	A. Control	9				71.0	336	73.3	456	138	18
				B. Lactate fed	10	80	60	75	71.1	638	73.4	498	138	27
				C. Lactate fed	11	170	108	64		1,500		502	165	26
Adrenalectomized	Insufficient Grollman extract intraperitoneally	Poor. Adrenal insufficiency	2	A. Control	8				73.5	102	75.9	425	61	28
				B. Lactate fed	9	170	62	31	72.1	163	74.3	451	98	131
Adrenalectomized	Reuben-Krick saline per os	Good	3	A. Control	12				72.8	50	75.7	406	76	65
				B. Lactate fed	10	115	66	57	72.8	177	73.5	442	121	72
Adrenalectomized	Sufficient Grollman charcoal adsorbate per os	Good	4	A. Control	8				72.4	79	74.7	434	60	26
				B. Lactate fed	7	110	64	58	71.7	525	74.2	510	136	79
				C. Lactate fed	6	170	113	66	71.4	916	74.9	510	139	87

similarly found a reduced rate of glucose absorption in fasting adrenalectomized rats. This reduced rate of absorption was the limiting factor in determining the maximum amount of lactic acid which could be introduced into the body successfully by way of the gastro-intestinal tract. The absorption of lactic acid was good in the salt-fed and charcoal-fed groups.

Normal animals. The normal fasting animal was in no acute distress for lack of carbohydrate, having appreciable reserves of glycogen still available in the liver, an adequate blood sugar, and no accumulation of lactic acid in the blood. Evidently the normal animal was able to use non-carbohydrate compounds during the fast to maintain a reserve of liver glycogen and blood sugar. The absorption of 60 mgm. of lactic acid by the normal 100 gram rat resulted in a deposition of glycogen in the liver, with no change in blood sugar, and no great accumulation of lactic acid in the blood.

Animals in adrenal insufficiency. The control group of fasted rats in adrenal insufficiency presented a picture of animals in obvious distress for lack of carbohydrate, the blood sugar being less than half that of the normal controls in spite of the hemoconcentration, and in spite of the fact that there was still some glycogen in the liver. The absorption of 62 mgm. of lactic acid brought about several changes, the most striking of which was the great accumulation of lactic acid in the blood. Obviously the liver failed to perform its function of removing lactic acid from the blood stream at the normal rate. That there was some conversion of blood lactic acid into liver glycogen, however, was attested by 1, the increased liver glycogen, and 2, the increased blood sugar. Changes in muscle glycogen were too small to be interpreted definitely as being significant, but were in the expected direction. In view of the fact that so much lactic acid remained unchanged in the blood stream and tissues, it is evident that the animal in adrenal insufficiency made some use of the small amount of added carbohydrate that was actually available to it, i.e., the portion that was successfully converted into liver glycogen. Unfortunately no carbohydrate balance could be calculated for these experiments because the usual assumptions on which such calculations are based obviously are invalid in these conditions. The salt-sustained animals, which to all appearances were normal, were found after fasting to be in a condition in some respects as precarious for lack of carbohydrate as those in adrenal insufficiency. Although the "clinical condition" of the two groups afforded the greatest contrast, their carbohydrate picture was similar inasmuch as the glycogen reserves were low and the utilization of the *d*-lactic acid was poor in both cases when compared with the normal. The blood sugar, however, was somewhat higher in the salt-sustained animals than in those in adrenal insufficiency. If this difference in blood sugar is significant it may account in part for the superiority of the salt-sustained

animals over those in insufficiency.[5] The fact that there was hemoconcentration in the animals in insufficiency in contrast to hydremia in the salt-sustained group makes comparisons of the composition of the blood of these animals precarious. The apparent blood lactic acid level of the lactate-fed animals was higher in animals in insufficiency than that in the salt-sustained animals. The striking contrast however lay between the relative failure of both the salt-fed animals and those in adrenal insufficiency to utilize the d-lactic acid as a source of liver glycogen and blood sugar as compared with the success of both the normal animals and the adrenalless animals sustained with charcoal adsorbate in utilizing the same compound.[6]

Animals protected by charcoal adsorbate. The fasted control group was characterized by low blood sugar and liver glycogen values, as compared with the normal control group in spite of the fact that the animals appeared to be in excellent condition and had access to the cortical hormone during the twenty-four hour fasting period.[7] In fact, the carbohydrate picture of this control group, as judged by the determinations made, appeared equally as critical as that in the control group sustained with salt or that in chronic insufficiency.

If the charcoal preparation were a complete substitute for the adrenal cortex, as revealed by studies of the endogenous metabolism, the carbohydrate stores of the control charcoal-fed animals (group 4A) should have been as good, or nearly as good, as those of the normal control group (1A). This proved not to be the case, and suggests the possibility that the cortical factor which is postulated by Long (13, 14) and Evans (15, 16), favoring the conversion of endogenous protein into carbohydrate, was lacking in this charcoal preparation, as it was in their cortical extracts. Also, the conditions affecting the lactate-fed groups may actually have been more rigorous than those affecting the controls inasmuch as the lactate was necessarily fed in a highly hypertonic solution, a condition which usually resulted in a slight dehydration of the tissues as measured by a

[5] The blood lactic acid value in the control group of salt-sustained animals was unexpectedly high. Whether this were true lactic acid or some organic compound simulating it by reacting with bisulfite cannot be stated. The latter seems probable because when lactic acid was ingested the apparent blood lactic acid value was only slightly increased above the value for the control group.

[6] It must be recognized that it is possible that better success in producing a normal utilization of d. lactic acid by salt-sustained animals might have been attained with other experimental conditions, such as with the use of a salt solution of different qualitative composition or greater concentration. There was no assurance in these experiments that the serum electrolyte pattern was strictly normal in spite of the normal appearance and behavior of the animals.

[7] Large quantities of charcoal were found in the gastro-intestinal tracts of these animals at the end of the experiment.

decrease in the total water content of the muscles and liver. Whether or not this small loss in water is accompanied by critical changes in the distribution of water and electrolytes between the cellular and extracellular fluids (as is the case after the repeated intraperitoneal injection of glucose into normal dogs (17), cannot be stated. When salt was given together with charcoal adsorbate to 6 animals no apparent benefits to the carbohydrate utilization were derived in comparison with the animals which were protected with charcoal adsorbate alone. Although the average figures for the carbohydrate values in the lactate-fed animals did not give a strictly normal picture in all respects, there was such a large and unequivocal change towards the normal that the results can be said to approach the normal. This becomes the more striking when the low values in this control group are taken into consideration. Obviously this adrenal cortical preparation enabled the adrenalless animals to store and to mobilize a potential source of carbohydrate (d. lactic acid) even though it did not prevent the carbohydrate stores of the body from being depleted during the 24 hour fast, under the conditions of these experiments. The experiments of Britton and Silvette (18) in which racemic lactate was given intraperitoneally[8] to adrenalectomized cats throw no light on the metabolism of the naturally occurring d. compound.

The efficacy of adrenal cortical preparations in facilitating the storage and mobilization of glucose has been demonstrated by Britton and Silvette (1, 20) Dambrosi (11) and Fernandez (21). Evans (16) inferred that the adrenal cortex of rats is concerned with the conversion of protein into carbohydrate. There is much evidence that the muscles of adrenalless animals contain less phosphocreatine (phosphagen) (22, 23, 24) and produce less lactic acid (25, 4, 26, 27) than do normal muscles.[9] That decreased lactic acid production may be a factor limiting the resynthesis of phosphocreatine in adrenalectomized animals was suggested by Buell, Strauss and Andrus (4).

SUMMARY AND CONCLUSIONS

1. The experiments described corroborated the hypothesis of Buell, Strauss and Andrus that the livers of animals in adrenal insufficiency fail to remove d. (sacro) lactic acid from the blood at a normal rate and to convert it into liver glycogen.

2. The rate of absorption of d. lactic acid from the gastro-intestinal tract of rats in adrenal insufficiency was definitely slower than in normal rats.

[8] The intraperitoneal injection of large quantities of glucose into a *normal* animal has been found to cause symptoms closely resembling those of adrenal insufficiency (19).

[9] Houssay (28) on the contrary found an increased lactic acid content in the resting muscle of the adrenalectomized rat and a greater increment in the lactic acid produced by such muscle after tetanization than by normal muscle.

3. Adrenalless rats, fasted for 24 hours, all showed low stores of carbohydrate in blood and liver whether they were in adrenal insufficiency or in good condition as a result of the protection afforded either by salt water or by an active charcoal adsorbate.

4. Adrenalless rats maintained in good condition by the use of salt drinking water failed to make normal use of ingested d. lactic acid.

5. Adrenalless rats maintained in good condition by the use of the charcoal adsorbate of Grollman, Firor and Grollman made excellent use of ingested d. lactic acid.

6. The errors in carbohydrate metabolism resulting from adrenalectomy do not *per se* necessarily cause the rapid death of the animal (rat) protected by salt from excessive loss of water and electrolytes.

REFERENCES

(1) Britton, S. W. and H. Silvette. This Journal **100**: 701, 1932.
(2) Loeb, R. F., D. W. Atchley, E. M. Benedict and J. Leland. J. Exper. Med. **57**: 775, 1933.
(3) Harrop, G. A., L. J. Soffer, R. Ellsworth and J. H. Trescher. J. Exper. Med. **58**: 17, 1933.
(4) Buell, M. V., M. B. Strauss and E. C. Andrus. J. Biol. Chem. **98**: 645, 1932.
(5) Buell, M. V. and M. B. Strauss. Bull. Johns Hopkins Hosp. **55**: 220, 1934.
(6) Grollman, A. and W. M. Firor. J. Biol. Chem. **100**: 429, 1933.
(7) Rubin, M. I. and E. T. Krick. Proc. Soc. Exper. Biol. and Med. **31**: 228, 1933.
(8) Grollman, A., W. M. Firor and E. Grollman. J. Biol. Chem. **110**: 189, 1935.
(9) Deuel, H. J., Jr., M. Gulick, C. F. Grunewald and C. H. Cutler. J. Biol. Chem. **104**: 519, 1934.
(10) Blatherwick, N. R., P. J. Bradshaw, M. E. Ewing, H. W. Larson and S. D. Sawyer. J. Biol. Chem. **111**: 537, 1935.
(11) Dambrosi, R. G., L. F. Leloir and A. Novelli. Compt. rend. Soc. biol. **114**: 1219, 1934.
(12) Cori, C. F. and G. I. Cori. J. Biol. Chem. **74**: 473, 1927.
(13) Long, C. N. H. Ann. Int. Med. **9**: 166, 1935.
(14) Long, C. N. H. and F. D. W. Lukens. Proc. Soc. Exper. Biol. and Med. **32**: 743, 1935.
(15) Evans, G. T. Proc. Soc. Exper. Biol. and Med. **32**: 1246, 1935.
(16) Evans, G. T. This Journal **114**: 297, 1936.
(17) Harrop, G. A., W. M. Nicholson and M. B. Strauss. Unpublished data.
(18) Britton, S. W. and H. Silvette. This Journal **107**: 190, 1934.
(19) Gilman, A. This Journal **108**: 662, 1934.
Darrow, D. C. and H. Yannet. J. Clin. Invest. **14**: 266, 1935.
(20) Britton, S. W. and H. Silvette. This Journal **100**: 643, 1932.
(21) Fernandez, R., V. G. Foglia, L. F. Leloir and A. Novelli. Compt. rend. Soc. biol. **115**: 334, 1934.
(22) Lang, K. Pflüger's Arch. **229**: 60, 1931.
(23) Ochoa, S. and F. Grande. Pflüger's Arch. **231**: 220, 1932.
(24) Cope, O., A. B. Corkill, H. P. Marks and S. Ochoa. J. Physiol. **82**: 305, 1934.
(25) Árvay, A. V. and L. Lengyel. Biochem. Ztschr. **239**: 128, 1931.
(26) Viale, G. Klin. Wchnschr. **12**: 467, 1933.
(27) Nachmansohn, D. J. Physiol. **81**: 36 P., 1934.
(28) Houssay, B. A. and P. Mazzoco. R. Soc. Arg. Biol. **3**: 491, 1927.

THE ELECTRIC PHENOMENA OF THE CRAYFISH CLAW DURING REPETITIVE STIMULATION

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The closing and opening movements of the crayfish claw are produced by two neuromuscular systems. Both the closing and opening muscles receive a double innervation of excitatory and inhibitory fibers, each characterized by distinct electric phenomena, as shown in a previous paper (du Buy, 1935). In order to investigate further the mechanism and interaction of these two systems, we have used the method of simultaneous stimulation of the two motor nerves by sine waves at various frequencies. By this method, one of us (Coppée, 1934) had previously been able to achieve stimulation which was selective according to frequency in the neuromuscular systems of frog and toad. Furthermore, we have recorded the electric responses of the opening and closing muscles, using stimulation by thyratron discharges at various frequencies, in order to study fatigue. We have also determined the velocity of the nerve impulse in both nerves in the intact animal and the corresponding neuromuscular delay.

TECHNIQUE. The sine waves were generated by a dynatron oscillator, and the R.M.S. voltage was measured by an A.C. voltmeter. The voltage was increased progressively by means of a precision potentiometer. The range of frequencies used was 20 to 2000 per second.

A thyratron circuit, driven either by a rotary interrupter for frequencies below 20 per second or by the dynatron oscillator for frequencies from 20 to 2000, permitted repetitive stimulation by brief shocks. The discharges of this circuit activated the primary of a Harvard inductorium. This procedure greatly reduced the stimulus artefact and obviated the necessity for special shielding or balancing out of the latter. The use of the thyratron eliminated confusion due to the differences between ordinary make and break shocks.

The stimulating electrodes (two silver wires chlorided by electrolysis) were inserted at various points in the basopodite and in the ischiopodite in the neighborhood of the two motor nerves, in order to stimulate both nerves simultaneously.

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As recording electrodes, we used either concentric silver needle electrodes, or two chlorided silver wires inserted in the opening or in the closing muscle.

The electrograms were recorded by the amplifier and the cathode ray oscillograph described by Garceau and Davis (1934).[3]

SINE-WAVE STIMULATION. It has been shown (Biedermann, 1888) that when the isolated nerve is stimulated by single shocks, the threshold of the opening neuromuscular system (excitatory for the opening muscle and inhibitory for the closing muscle) is lower than that of the closing neuromuscular system (excitatory for the closing and inhibitory for the

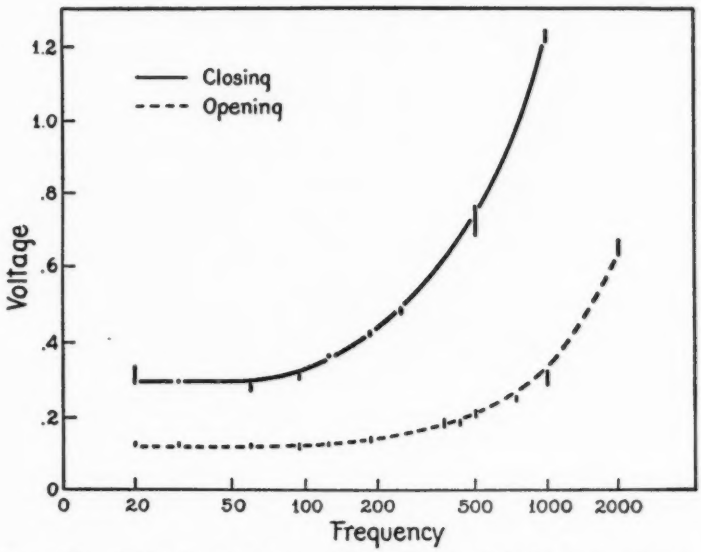

Fig. 1. Thresholds of opening system (broken line) and closing system (continuous line) for electrical stimulation at various frequencies of sine waves.

opening muscle). In the intact animal the relative thresholds depend also on the exact positions of the stimulating electrodes in the tissues. The most favorable location was determined by trial in each case.

In most cases, we were able, on progressively increasing the stimulating current, to obtain first the threshold for the opening movement, and then, by further increase, the threshold for the closing movement. Both curves

[3] The original construction and subsequent development of this apparatus was assisted by grants from the DeLamar Mobile Research Fund of the Harvard Medical School, from the Josiah Macy, Jr. Foundation, from the American Otological Society and from several anonymous donors.

in figure 1 were obtained in this way on the same claw without changing the position of the electrodes. For each frequency, the thresholds for opening and closing were checked alternately two or three times, in order to be sure of the constancy of the measurement. The variations were small, usually less than 5 per cent. This contrasts with the extreme variability of the rheobase determined with a constant current of long duration.

As shown in figure 1, the type of liminal-voltage frequency curve differs in the two systems. The curve for the closing neuromuscular system resembles the curves previously observed by one of us (see Coppée, 1934), using the same method of stimulation, on the sciatic nerve, gastrocnemius and sartorius of *Rana* and *Bufo* and on the foot of *Helix*; i.e., after a rather flat portion for frequencies below 100 c.p.s., showing a more or less definite minimum, the threshold voltage rises as the frequency increases. The dynatron oscillator did not provide pure sine waves below 20 c.p.s. We were therefore unable to examine the thresholds at lower frequencies. On the other hand, the curve for the opening neuromuscular system shows that the threshold is altered very little by increasing the frequency of the stimulating current except at frequencies above 500 c.p.s. Considering the two types of curves and the relative thresholds for each frequency in the two systems, it is possible that a favorable electrode placement in the basopodite would yield a lower threshold at low frequencies for the closing than for the opening system. In some cases, by progressively increasing the intensity of the current, we succeeded in getting, for low frequencies, the closing threshold only, and for higher frequencies the opening threshold first. Thus by using two different frequencies, we had achieved the selective stimulation of the complex mechanism of the claw. One of us (G. C.) has also found a similar selective stimulation in partially injured frog nerves when the strength-frequency curve of one bundle is flattened as a result of the lesion, and the rest remains unchanged. But, while it was possible with the high frequencies to get closing by increasing the current above the opening threshold, we never succeeded at low frequencies in observing an opening when we increased the intensity above the closing threshold. This can be understood on the basis of the known differences between the two systems, i.e., lower threshold for the opening system, and greater force of the closing muscle. Once a closing stimulus was given, the opening muscle could not overcome the contraction of its stronger antagonist. Moreover, in this case the inhibitory fibers to the opening system are stimulated too, as one of us has already shown (du Buy, 1935).

Mechanical response. In these experiments, we have always considered as threshold the smallest movement of opening and closing of the dactylopodite. All records show first a fall and then a rise as the stimulus is

progressively increased. For low frequencies (below 180 c.p.s.) the response is a continuous tetanus without any relaxation (see fig. 2A); above 180 c.p.s. for the closing system and above 250 for the opening system, the tetanus is interrupted from time to time by partial relaxation, as shown in figure 2B, C, D. In order to interpret these two types of mechanograms, we tried to study the electric response of the muscle, but this was impossible because of the tremendous electric artefacts. Therefore, we employed the thyratron oscillator and inductorium at similar frequencies in order to avoid this difficulty.

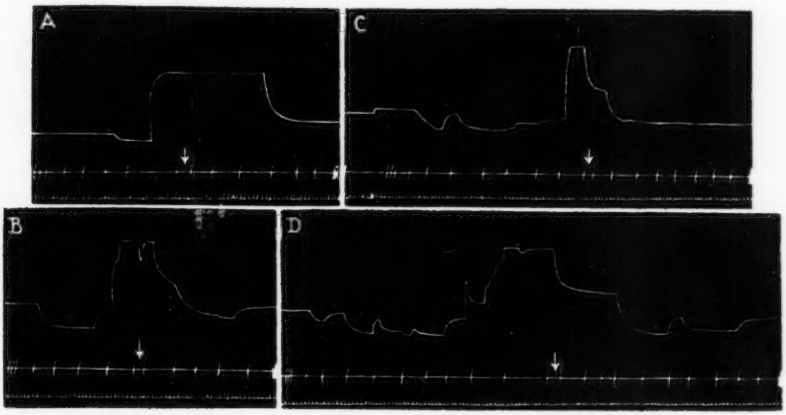

Fig. 2. Mechanogram of the movements of the dactylopodite. Continuous stimulation of all nerve systems in coxopodite. The stimulus is increased at each signal up to the one designated by the arrow. Thereafter it is progressively decreased. A, 30 c.p.s.; B, 188 c.p.s.; C, 375 c.p.s.; D, 500 c.p.s. Time interval = 1 second. Closing of the claw corresponds with an upward deflection of the record. All records show first a fall corresponding with opening of the claw.

REPETITIVE STIMULATION BY BRIEF SHOCKS. In order to show that this type of stimulation does not differ appreciably from the previous method, threshold values for the closing system, similar to those plotted in figure 1, are given in table 1. These observations also prove that the mechanical and electrical thresholds (i.e., the just-visible movement of the dactylopodite and the first appearance of the action potentials) are the same. Figure 3A shows a well-sustained electric response of the opening muscle to each thyratron discharge at 94 c.p.s.; no alternation takes place at this frequency. During a long period of stimulation, the shape and size of the action potentials remained practically constant. Figure 3B shows the corresponding response for the closing system. Although both systems follow this frequency, the amplitude of the action potentials

of the closing muscle decreases very rapidly. The more rapid decrease in the closing muscle at this frequency (fig. 3B) cannot be explained by assuming that some motor units cease to respond, because (1) concentric electrodes recording from a few muscle fibers gave similar responses, and (2) the fatigue progresses regularly and only the amplitude of the action potential changes. Moreover, the action potentials of the closing muscle do not follow the stimuli to such high frequencies as the opening muscle, as is shown in figure 3; in which A, C, E, G, and H show the action potentials of the opening muscle at 94, 188, 375, 435, and 500 c.p.s.; B, D and F, the action potentials of the closing for the frequencies 94, 188, and 375 c.p.s.

The opening muscle usually follows the rhythm of stimulation by responding to each shock, to one in every 3 (fig. 3E, G), 4 (fig. 3E, G), or 6

TABLE 1

Recording electrodes in closing muscle

FREQUENCY	DIAL POSITION		FREQUENCY	DIAL POSITION	
	Threshold action potential	Threshold for closing		Threshold action potential	Threshold for closing
c.p.s.			*c.p.s.*		
30	12	12	500	42	42
60	14.5	14.5	625	50	50
94	22	22	750	65	65
125	25	25	875	77	77
188	27	27	1000	94	94
375	32	32	30	11.5	11.5
435	37.5	37.5	435	37.5	37.5
			500	42.5	42.5

The observations were made in the order given.

shocks (fig. 3H), or by changing during a given period of stimulation. The closing muscle, however, reacts more casually. This explains the erratic mechanograms of figure 2. The closing system differs from the opening muscle (1) by fatigue (or equilibration) at lower frequencies; (2) by not following higher frequencies regularly. Such a difference of reaction to frequencies above 200 per second might be explained by a difference in some chronological characteristic such as the refractory period.

NEUROMUSCULAR DELAY. The neuromuscular delay was measured in two ways: 1. The nerves were stimulated at two different points in the basopodite and meropodite. The time difference between the electric artefact and the foot of the action potential for each stimulated point allowed us to determine the velocity of the impulse in both nerves (fig. 4e-f). Furthermore, assuming that the velocity remains the same for

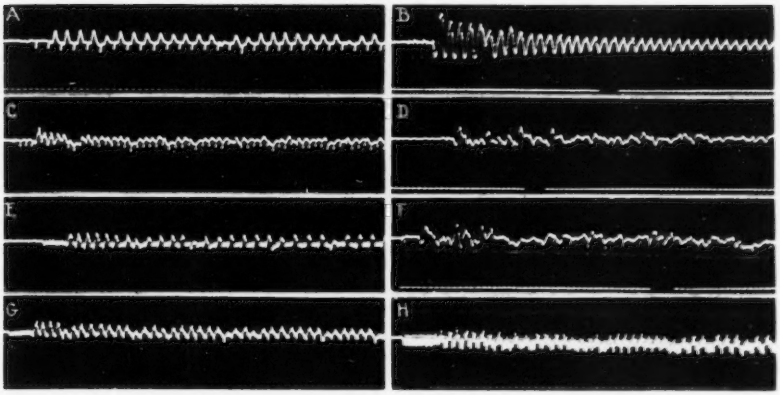

Fig. 3. A, C, E, G, H: electrograms of opening muscle at 94, 188, 375, 435, and 500 c.p.s. B, D, F: electrograms of closing muscle at 94, 188, and 375 c.p.s. Time interval = 5 msec.

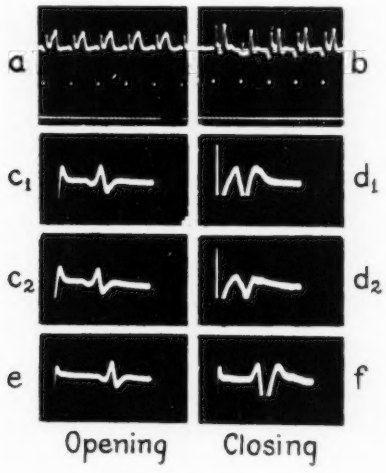

Fig. 4. Standing waves, 30 stimuli per second. Distance between stimulating electrodes = 175 mm. Distance between distal stimulating electrodes and first recording electrode = 15 mm. Electrograms: a, direct stimulation of opening muscle; b, direct stimulation of closing muscle; c_1, c_2, stimulating electrodes proximal, record of opening muscle. Time interval between c_1 and c_2 about 15 seconds; no fatigue. e, stimulating electrodes distal, record of opening muscle. d_1, d_2, stimulating electrodes proximal, record of closing muscle (beginning of stimulus artefact barely visible). Time interval between d_1 and d_2 about 15 seconds; fatigue. f, stimulating electrodes distal, record of closing muscle.

the total length of the fiber, it is possible to determine the time required for the impulse to reach the recording electrode. The time difference between the latency of the muscular response and the calculated conduction time in the nerve measures the neuromuscular delay.

2. Direct stimulation of the muscle showed a latency between the artefact and foot of action potential of the same magnitude as the value determined by the other method. We infer that in this case it is really the intramuscular nerve fibers that are stimulated. Both the closing and the opening muscles were stimulated in this way. Figure 4a shows the results for the excitatory impulse of the opening muscle; figure 4b, for the closing muscle. The data obtained from this figure are given in table 2.

The above experiment is a typical one. The absolute values, however, vary from one animal to another. Differences in temperature and the degree of fatigue are important causes of variability. Nevertheless, in all experiments the velocity of conduction of the opening nerve system is

TABLE 2

	CONDUCTION VELOCITY	NEUROMUSCULAR DELAY
	m. p. s.	*msec.*
Excitatory impulse of opening system	2 6	4 1
Excitatory impulse of closing system	4 25	3 4

within 60 to 85 per cent of the velocity of conduction of the closing nerve system and remains constant during an experiment.

The neuromuscular delay of the opening system is always found longer than of the closing system in *fresh* animals. After repeated stimulation the neuromuscular delay of both systems is lengthened. Although the opening system has a longer neuromuscular delay, and a lower velocity of conduction, it follows, however, the rhythm of a higher frequency of stimulation, as was shown on page 286. It therefore seems that the opening system, although it shows a longer neuromuscular delay, has a shorter refractory period.

SUMMARY

Stimulation of the motor nerves of the crayfish claw by sine waves or by repetitive shocks at various frequencies shows that:

1. The threshold for the opening system remains practically constant up to 1000 c.p.s.

2. The threshold for the closing system is increased considerably by increasing the frequency of stimulation. The threshold in both cases was determined by mechanograms and by electrograms.

3. The opening system follows the frequencies above 80 c.p.s. by alternation; the closing system reacts irregularly.

4. The velocity of nerve conduction of the opening system is about 3 meters per second; that of the closing system about 4 meters per second. The neuromuscular delay of the opening system in fresh animals varies from 4 to 6 msec.; that of the closing system from 3 to 4 msec. The neuromuscular delay of the closing system is always the shorter in a given preparation.

5. The closing system is more susceptible to fatigue than the opening system. It is inferred that the refractory period of the opening system as a whole is shorter than that of the closing system, in spite of its longer neuromuscular delay and slower velocity of conduction.

REFERENCES

BIEDERMANN, W. Sitzungsb. Akad. Wiss. Wien **96:** 1888.
BUY, H. G. DU. This Journal **114:** 224, 1935.
COPPÉE, G. Arch. Internat. Physiol. **38:** 251, 1934.
GARCEAU, E. L. AND H. DAVIS. This Journal **107:** 305, 1934.

THE EFFECT OF PROLONGED ANOXEMIA ON THE HEART AND SPLEEN IN THE MAMMAL

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Strohl in 1910 reported that the heart weight-body weight ratio of the alpine snow bird (*Lagopus alpinus Nilss*) was much greater than that of a very near relative, the moor snow bird (*Lagopus albus Gmelin*). He concluded that this greater heart weight in the former was due to two factors, namely, a work hypertrophy and a specific adaptation to the rarefied atmosphere of the mountainous habitat.

In view of the controversy over the causes of cardiac hypertrophy it was deemed worth while to study Strohl's observations further and to attempt to produce experimental cardiac hypertrophy by subjecting animals to low oxygen tensions.

METHODS. Young adult female guinea pigs were chosen for this work. The latest significant work on the heart weight-body weight ratio in these animals was reported by Joseph in 1908. His animals, however, had been used for other purposes and since he reported on a small series it was necessary to establish a normal figure for this work. Consequently the ratio between the heart weight and body weight was determined in 71 normal female guinea pigs (Van Liere and Sleeth, 1936). The animal was weighed and then killed by a blow on the head. The thorax was opened and the heart removed from the pericardium. The great vessels were trimmed flush with the heart and all the chambers were opened and washed free of blood. The excess moisture was removed by filter paper and the organ carefully weighed. The ratio was determined by dividing the heart weight in grams by the body weight in grams, expressing the result in per cent. The ratios as expressed in this paper, therefore, represent the number of grams per cent of heart per kilogram body weight.

No figures were available on the normal spleen weight in the guinea pig. Since it was felt that anoxemia might have an effect on the weight of the spleen, the ratio between the spleen weight and the body weight was determined in a similar manner in a series of 43 normal animals.

Twenty vigorous young adult female animals were chosen to be subjected to anoxemia. These animals were placed in a steel respiratory

chamber previously described (Van Liere, 1927). The interior of this chamber was kept at a pressure varying between 440 and 380 mm. Hg. These figures correspond to an oxygen percentage of 12.25 to 10.50 and to an altitude range of 14,000 to 18,000 feet. Adequate ventilation was provided so that carbon dioxide could not accumulate. The animals were kept under these conditions constantly except for about 20 minutes a day (necessary to clean the chamber and care for the animals) for periods from 20 to 105 days.

RESULTS. Table 1 summarizes briefly the results obtained. It will be seen that exposure to prolonged anoxemia produces very definite effects upon the weight of the heart and spleen. The heart weight-body weight ratio increased in the anoxemic animals to 155.8 per cent of the normal value. The spleen weight-body weight ratio increased to 531.0 per cent of the normal value.

TABLE 1

	HW:BW RATIO					SW:BW RATIO				
	Number of animals	Mean	Median	Mode	Per cent*	Number of animals	Mean	Median	Mode	Per cent*
Normal	73	0.319	0.309	0.309	100	43	0.156	0.145	0.145	100
Anoxemia	15	0.496	0.447	0.443	155	9	0.828	0.804	0.8	531

*Per cent of normal mean.

Table 2 summarizes the work done upon the 15 animals. Unfortunately, the data on 6 spleens were lost, so that only 9 are reported. Five of the animals died; the data obtained from these animals were not used.

DISCUSSION. In studying table 2 it will be seen that 5 animals gained weight, one an unappreciable amount, but the remaining 4 gained from 6.18 to 26.45 per cent. In spite of this gain in weight 4 out of these 5 animals showed a relative increase in cardiac weight.

Although these animals received the best of care and the choicest food, 5 out of 20 died. Ten of the remaining 15 lost a good deal of weight — from 1.36 to 29.40 per cent. The loss of weight was due to the fact that the majority of the animals did not eat well under low oxygen tensions. A number of standard text books in physiology still state that the heart loses very little weight during inanition. This is a misconception as it has been shown by Chossat (1843), Manassein (1860), Lazareff (1895) and Jackson (1915), that the heart loses proportionally as much weight during inanition as does the rest of the body. Recent work by Van Liere and Sleeth (in press) on the guinea pig confirms the earlier work.

The fact that the organ weight-body weight ratios do not change materially in inanition is a very important consideration as far as the results re-

ported in this paper are concerned. It is obvious that if the heart did not lose proportionally as much weight as does the body, the findings reported in this paper would have no real significance.

Of the 15 animals which survived the experiment and were killed and autopsied in the usual manner, the heart and spleen of 4 representative specimens were examined microscopically. The examinations revealed definite cardiac hypertrophy in 3 cases. One heart showed signs of atrophy but gave evidence of previous hypertrophy. This particular animal had been kept under anoxemic conditions for 105 days and one might venture to suggest that atrophy followed the primary hypertrophy. The spleens all showed marked phagocytosis of red blood cells and many

TABLE 2

NUMBER	DAYS IN CHAMBER	PER CENT CHANGE IN BODY WEIGHT	HW:BW RATIO	PER CENT CHANGE IN HW:BW RATIO	SW:BW RATIO	PER CENT CHANGE IN SW:BW RATIO
1	20	−11.93	0.5390	+69		
2	22	+26.45	0.3873	+21	1.5747	+910
3	22	−24.37	0.6272	+96		
4	25	−13.44	0.5140	+61	1.1376	+629
5	35	+6.18	0.3857	+21		
6	44	−34.12	0.6455	+101	0.9964	+538
7	44	−1.36	0.6368	+99		
8	40	−31.43	0.3909	+22	0.8045	+415
9	45	+20.79	0.2986	−6		
10	48	−17.94	0.4430	+39		
11	50	+12.47	0.4437	+39	0.6352	+307
12	53	+1.50	0.7483	+134	0.5566	+257
13	62	−18.42	0.4473	+40	0.3784	+142
14	77	−26.56	0.5138	+61	0.7450	+378
15	105	−29.40	0.4239	+33	0.6271	+302

phagocytes showed hemosiderin. In one case the sinuses of the spleen were greatly dilated and engorged with erythrocytes.

It is known that high altitudes cause a marked polycythemia and thus many red blood cells would presumably have to be destroyed. This increased cell destruction which the spleen would be called upon to perform might well account for the greatly enlarged spleens which these animals had.

It is more difficult to account for the cardiac hypertrophy. As previously mentioned, Strohl thought that the cardiac hypertrophy in the alpine snow bird was due to the increased work of the heart and a specific adaptation to the rarefied atmosphere. Some physiologists, however, are reluctant to admit that there is a work cardiac hypertrophy. These men, on the other hand, believe that cardiac hypertrophy is always preceded

by some injury to the cardiac muscle fiber. Unfortunately, the experiments reported here do not throw much light on this controversial subject as neither factor can be ruled out. It is quite possible that both factors may be at work here. Under low oxygen tensions the heart beats faster and this probably increases the amount of work it has to do. The increased viscosity of the blood would also throw a greater load on the heart. On the other hand, anoxemia may have deprived the heart of sufficient oxygen and consequently the cardiac fibers were actually injured. Kaufmann and Meyer (1917) studied the hearts of soldiers who had returned from campaigns in mountainous districts and found that the hearts of these soldiers were greatly increased in size. These enlarged hearts, however, decreased in size after a short rest. It was shown by Takeuchi (1925) by opening the chest wall and using the cinematograph, and Van Liere (1927) by use of the x-ray in the normal animal that anoxemia can produce cardiac dilatation. In order to produce it, however, severe degrees of anoxemia must be used. The author feels that with the animals at rest it is very problematic whether 10 or 12 per cent of oxygen would produce cardiac dilatation.

The question of inanition arises; it is possible that this could produce cardiac dilatation which subsequently could be followed by hypertrophy. But a number of the animals gained weight so that this factor must not be stressed too greatly.

SUMMARY AND CONCLUSIONS

Fifteen guinea pigs were kept at low oxygen tensions for periods varying from 20 to 105 days. The oxygen percentages ranged from 12.25 to 10.50 per cent. It was found that the average heart weight-body weight ratio was increased to 155.8 per cent of the normal value. The average spleen weight-body weight ratio in 9 animals increased to 531.0 per cent of the normal value.

Owing to the polycythemia caused by the low oxygen tensions the spleen would have to destroy many red blood cells and it is suggested that this might well account for the increased size of this organ.

The hypertrophy of the heart could have been caused either by a work hypertrophy or an injury hypertrophy or perhaps a combination of both factors.

The work reported in this paper lends experimental evidence to the observations made by Strohl that rarefied atmosphere is capable of producing cardiac hypertrophy.

I wish to express my thanks to Dr. C. H. Bunting of the University of Wisconsin for making the microscopic examinations of the tissues reported in this work.

REFERENCES

CHOSSATT, C. Savants Etrangers, Paris **8:** 1843.
JACKSON, C. M. Am. J. Anat. **18:** 75, 1915.
JOSEPH, D. R. J. Exper. Med. **10:** 521, 1908.
KAUFMANN, H. AND H. H. MEYER. Med. Klinik. **44:** 45, 1917.
LAZAREFF, N. Dissertation, Warsaw, 1895.
MANASSEIN, W. Centralbl. f. d. Med. Wissenschaft. **6:** 273,1868.
STROHL, J. Zool. Jahrb. Jena **30:** 1, 1910.
TAKEUCHI, T. J. Physiol. **60:** 282, 1925.
VAN LIERE, E. J. This Journal **82:** 727, 1927.
VAN LIERE, E. J. AND C. K. SLEETH. Proc. Soc. Exper. Biol. and Med. **34:** 41, 1936.

THE RELATION BETWEEN RHYTHMIC VARIATIONS IN BLOOD PRESSURE AND RHYTHMIC CONTRACTIONS OF THE ARTERY OF THE EAR OF RABBITS AND DOGS

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Spontaneous rhythmic contractions in mammalian arteries were first described by Schiff (1854) who observed them through the intact skin in the ears of unanesthetized rabbits. Clark and Clark (1932) and Wilson (1936) have studied this phenomenon under the microscope in the preformed transparent chamber in the rabbit's ear.

Waves are occasionally observed on normal blood pressure records which are much slower than respiratory waves. These are usually referred to as "Traube-Hering waves" (Traube, 1865; Hering, 1869), though more correctly as vasomotor waves of blood pressure, since Traube's and Hering's waves were obtained on curarised animals under artifical respiration.

The present study is an attempt to find out what relation, if any, exists between the rhythmic contractions of the main artery of the ear and the vasomotor waves of blood pressure.

METHODS. *Observations on the ear.* Experiments were carried out on six rabbits and five dogs, the rabbit experiments at the University of Edinburgh and the dog experiments at the University of Pennsylvania. Animals were chosen whose ears were white, and as thin as possible, in order that the main artery could be clearly seen through the intact skin.

For preliminary observation of the ear before the blood pressure experiments were carried out, rabbits were placed in a box and dogs were trained to sit on a table or lie quietly on an animal board for observation. Every precaution had to be taken not to disturb the animal since the artery becomes greatly constricted and rhythmic contractions cease. Animals which are very phlegmatic and prone to go to sleep are unsatisfactory, since sleep is inhibitory to rhythmic contractions (Clark and Clark, 1932).

Blood pressure. Previous work (Wilson, 1936) indicated that rhythmic contractions were inhibited by anesthetics (ether, chloroform, luminal Na, Na amytal). The use of morphine as a preliminary to anesthesia was suggested, since Traube-Hering waves can sometimes be elicited by morphine (L. Hill, 1896).

Blood pressure records were made in the usual way, with a mercury manometer. In the case of the rabbits, the experiments were carried out under chloroform, following large doses of morphine, and the blood pressure was taken in the carotid artery.

As it was difficult to get dogs suitable for the observations on the ear, it was advantageous to use one dog for more than one experiment, if possible. A sterile operation[1] to externalize the femoral artery was performed under Na amytal anesthesia. The artery was exposed for as great a length as possible, the cut edges of the muscle sewed together under the artery and the wound closed by drawing the skin together with loose stitches. On a subsequent day a large dose of morphine was injected subcutaneously and the region around the externalized femoral artery was well infiltrated with 0.5 per cent panthesin (Sandoz) or 0.1 per cent cocaine HCl. The stitches in the skin could then be clipped and the cannula inserted in the artery as peripherally as possible, so that the artery could be used again. Blood pressure records could then be made for two or three hours with little or no discomfort to the animal.

The ear was watched while the blood pressure was being recorded and the onset of each contraction and dilatation observed in the main artery of the ear was recorded on the drum. In many experiments respiration was also recorded.

At the close of an experiment the artery was tied off, and the wound closed. No more than four experiments were ever performed on one dog.

EXPERIMENTAL. *The effect of morphine and anesthetics on rhythmic contractions.* A few experiments carried out previously (Wilson, 1936) indicated that anesthetics were depressant to rhythmic contractions in the artery of the rabbit's ear. Further experiments amply confirmed this. Ether, chloroform, Na amytal and urethane were usually inhibitory, though chloroform was the least depressant to rhythmic contractions. Morphine was never found to be inhibitory and often increased the rate of rhythmic contractions, especially in the stage when the blood vessels were dilated. The dilatation of the blood vessels is not in itself responsible for the increase in rhythmic contractions. They do not appear when the blood vessels are dilated under various other conditions (Wilson, 1936). The first parts of figure 1 (a) and (b) show the stimulating effect of morphine: two experiments on rabbit 1 with doses of 13 mgm. per kilo. (The animal had received on previous days three injections of morphine of 20 mgm. per kilo each.) The latter parts of the curves show the inhibitory effect of ether and chloroform.

Blood pressure experiments. Examples of experiments in which general

[1] The author wishes to acknowledge the help and advice of Dr. Charles G. Johnston of the Department of Research Surgery of the University of Pennsylvania in the operation for externalizing the artery.

blood pressure and rhythmic contractions in the main artery of the ear were simultaneously recorded are given in figure 2 (a) to (h), tables 1 and 2 and protocol 1.

In some of our experiments no rhythmical arterial contractions were present, especially in the rabbit experiments where chloroform was used. Usually when contractions were present there were corresponding waves on the blood pressure record. As will be seen from the sample tracings given in figure 2 there was great variety in size, shape and rate of these blood pressure waves. In every experiment the blood pressure waves and the rhythmic contractions in the ear were much slower than the

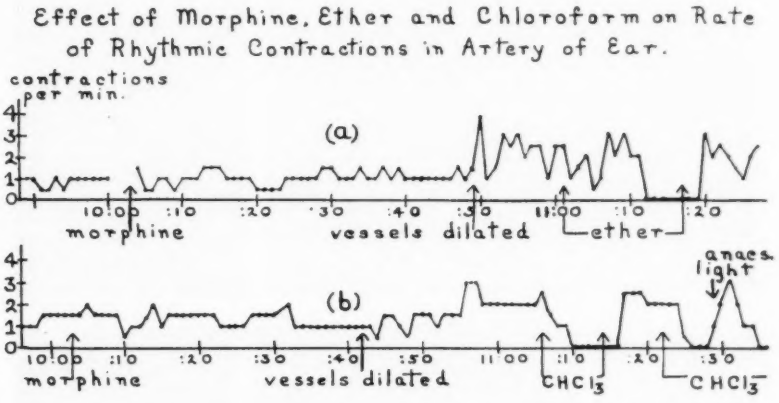

Fig. 1. Rabbit 1. Two experiments on consecutive days. Abscissae: Time in hours and minutes, 10 to 11:30 a.m.

respiration, but there was a correspondence in a very few experiments (fig. 2 (d), and in part of fig. 2 (a)) between the rate of the rhythmic contractions in the ear and respiratory gasps, *R*, occurring periodically after several normal respirations. In experiments such as those shown in figure 2 (c), (e) and (f) where the respiration was uniform there appears to be no relation whatever between the respiration and the vascular phenomena. In figure 2 (g) and (h), although the respiration was irregular there was no correspondence with the larger blood pressure waves. Irregular respiration and variations in heart rate did, of course, affect the blood pressure and often the variations were great enough to interfere with the clearness of the slower waves. In an attempt to smooth out such interferences we tried injections of atropine. Figure 2 (e) shows a record taken ½ hour after atropine, when the heart rate was approximately 200 and the respiration 30 per minute. The marked waves of blood pressure

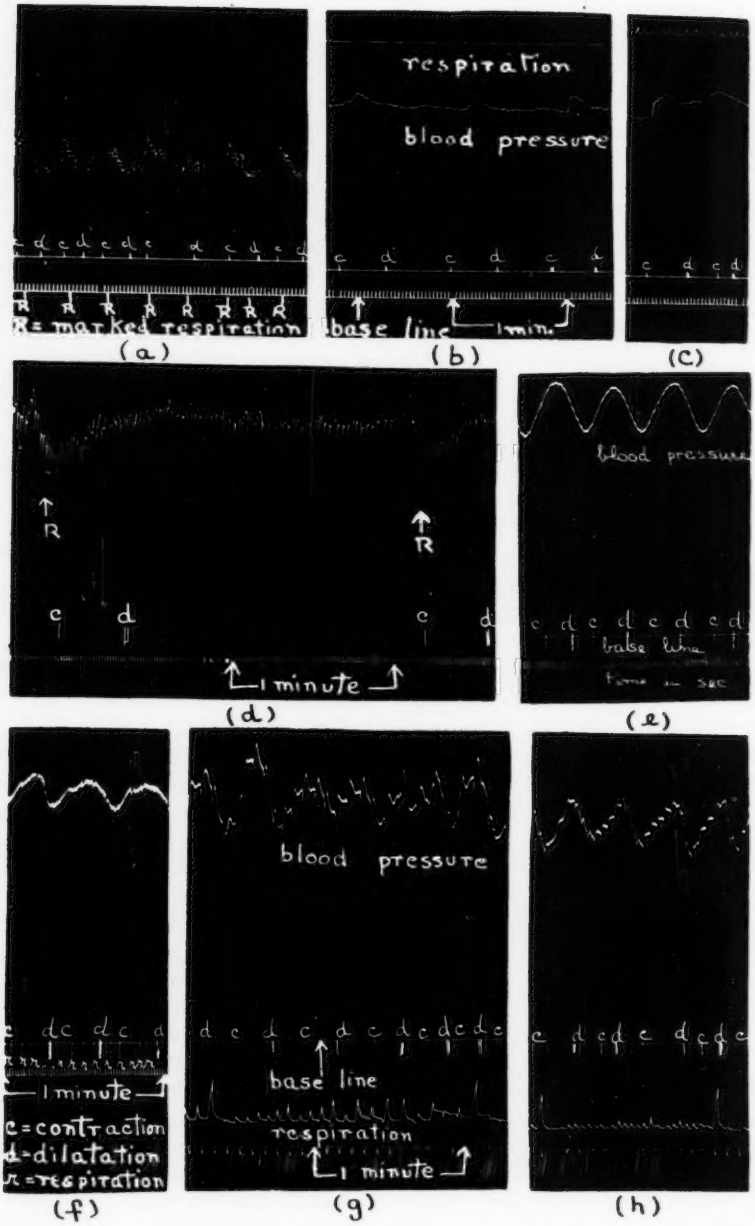

Fig. 2. Simultaneous records of blood pressure and rhythmic contractions in main artery of ear. c = contraction; d = dilatation; r = respiration; R = marked respiration. (a), rabbit 2, light $CHCl_3$ anesthesia; (b) and (c), rabbit 3, light $CHCl_3$ anesthesia, see table 1; (d)–(h), dog 1, local anesthesia, 2 experiments, see protocol 1 and table 2.

TABLE 1

Rabbits 2 and 3, morphine 20 mgm. per kilo (1st dose) light chloroform anesthesia

	FIGURE 2 (A)	FIGURE 2 (B)	FIGURE 2 (C)
Rhythmic contractions per minute	3	1	2
Blood pressure waves per minute	3	1	2
Respiration per minute	9–12	20	15
Marked respiration per minute	3	0	0
Heart rate per minute	100±	150–200	150–200
Mean blood pressure mm. Hg	100–72	126–116	130–114

Protocol 1. Dog 1. White Poodle. Weight 8 kilo

12/11 Sterile operation under Na amytal, left femoral artery externalized by sewing muscle beneath artery and skin above.

12/22 Sterile operation under Na amytal, right femoral artery externalized.

1/6 Wounds well healed. Animal acclimatized to procedure by previous experiments.

10:36 a.m. 4th dose morphine (subcut.), 8.5 mgm. per kilo (1st dose 12/16)
11:56 a.m. Blood pressure in right femoral recorded.
12:26 p.m. Record shown in figure 2 (d)
1:40 p.m. Subcutaneous injection of atropine sulfate (0.125 mgm. per kilo)
2:17 p.m. Record shown in figure 2 (e)
2:59 p.m. Record shown in figure 2 (f)

1/10

10:45 a.m. 5th dose of morphine (now increased to 12.5 mgm. per kilo) Unsuccessful attempt to use left femoral artery so right carotid cannulated (ether administered for operation).
2:40 p.m. Blood pressure recorded.
2:46 p.m. Ether discontinued.
2:58–9 p.m. Vagi cut in neck, ether administered.
3:07 p.m. Atropine sulfate injected subcutaneously (0.125 mgm. per kilo)
3:15 p.m. Marked waves appear on blood pressure record.
3:37 p.m. Record shown in figure 2 (g)
3:53 p.m. Record shown in figure 2 (h)

TABLE 2

Data of protocol 1: 1st experiment (d), (e), (f); 2nd experiment (g), (h)

	FIGURE 2 (D)	FIGURE 2 (E)	FIGURE 2 (F)	FIGURE 2 (G)	FIGURE 2 (H)
Rhythmic contractions per minute	½	3	3	3	3
Blood pressure waves per minute	½	3	3	3	3
Respiration per minute	24–28	30	13	12	21
Marked respiration per minute	½	0	0	1	1
Heart rate per minute	120±	200±	140	200±	200±
Mean blood pressure mm. Hg	140–94	156–122	162–138	180–120	150–144

began in less than $\frac{1}{4}$ hour after the atropine and continued for $1\frac{1}{2}$ hour. In (f), taken $1\frac{1}{4}$ hour after (e), the heart rate is 140 showing that the atropine effect on the heart, at least, has worn off. The waves are not so great, but are still marked. In another experiment the vagi were cut without causing any marked waves on the blood pressure record. Subsequently atropine was given and marked waves appeared in 8 minutes (fig. 2 (g) and (h), $\frac{1}{2}$ and $\frac{3}{4}$ hour after atropine). In some experiments, however, there were no marked blood pressure waves after atropine, so it is impossible to tell what rôle, if any, atropine played in the production of the very marked waves in figure 2 (e).

Discussion. There is considerable difference of opinion in the literature as to whether rhythmical contractions in arteries and undulatory waves of blood pressure are of central or peripheral origin. There is some evidence that spontaneous contractions in the rabbit's ear cease when the ear is denervated (Lewin and Schilf, 1927). Many of the older experiments which pointed to a local origin are rendered inconclusive by recent evidence that complete denervation was not accomplished by the operative procedures (Grant and Bland, 1932).

Blood pressure waves, such as are found in our experiments, have usually been considered as of central origin (Roy, 1880), though Barcroft, Nisinaru and Steggerda (1932) believe that "the rhythm itself is peripheral and is a property of the muscular, or neuro-muscular complex."

In all the experiments in this study, whenever there were definite waves on the blood pressure record there were, synchronously, rhythmic contractions in the main artery of the animal's ear. During the rise in blood pressure the artery was constricted. If the changes in the ear were passive, the artery would have been dilated. It is impossible that these changes in the arteries of the ears alone could account for changes in general blood pressure of the magnitude shown here (sometimes 40 mm. Hg). Several investigators have found that vasomotor changes in various organs caused undulatory changes in blood pressure of rhythm similar to that described in this paper. The experiments of Roy (1880) were on the kidney and spleen and of Barcroft and co-workers (1932a, b, c, d) on the spleen and intestine. Probably simultaneous rhythmic changes in blood vessels in various parts of the body are the cause of the blood pressure waves we have described. This would rule out a local origin for these phenomena and we have shown above that respiratory changes are not the causative agent. This points to the vasomotor change as the origin of these changes in the vessels of the ear and in the blood pressure, though our experiments do not rule out the possibility of some chemical agent being responsible for these rhythmic changes.

SUMMARY

Simultaneous records have been made of blood pressure and rhythmic contractions of the main artery of the ear in rabbits and dogs. When vasomotor waves appear on the blood pressure record they are synchronous with rhythmic contractions and dilatations in the artery of the ear. These vasomotor changes in the ears are undoubtedly only part of the vascular changes in the body which are responsible for the blood pressure variations.

Studies were made on the inhibitory effect of anesthetics and the stimulating effect of morphine on rhythmic contractions.

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REFERENCES

BARCROFT, J., L. C. KHANNA AND Y. NISIMARU. J. Physiol. **74:** 294, 1932.
BARCROFT, J. AND Y. NISIMARU. J. Physiol. **74:** 299, 1932.
J. Physiol. **74:** 311, 1932.
BARCROFT, J., Y. NISIMARU AND F. R. STEGGERDA. J. Physiol. **74:** 490, 1932.
CLARK, E. R. AND E. L. CLARK. Am. J. Anat. **49:** 441, 1932.
GRANT, R. T. AND E. T. BLAND. Heart **16:** 69, 1932.
HERING, E. Sitzungsb. d. K. Akad. d. Wissensch., Wien **60:** 839, 1869.
HILL, L. The physiology and pathology of the cerebral circulation. J. and A. Churchill, London, 1896.
LEWIN, H. AND E. SCHILF. Pflüger's Arch. **216:** 657, 1927.
ROY, C. S. J. Physiol. **3:** 203, 1880.
SCHIFF, M. Arch. f. Physiol., Heilk **8:** 523, 1854.
TRAUBE, C. Centralbl. f. d. med. Wissensch., Berlin, S. 881, 1865.
WILSON, H. C. J. Pharmacol. Exper. Therap. **56:** 97, 1936.

ALTERATIONS IN THE ELECTRICAL FIELD PRODUCED BY CHANGES IN THE CONTACTS OF THE HEART WITH THE BODY[1]

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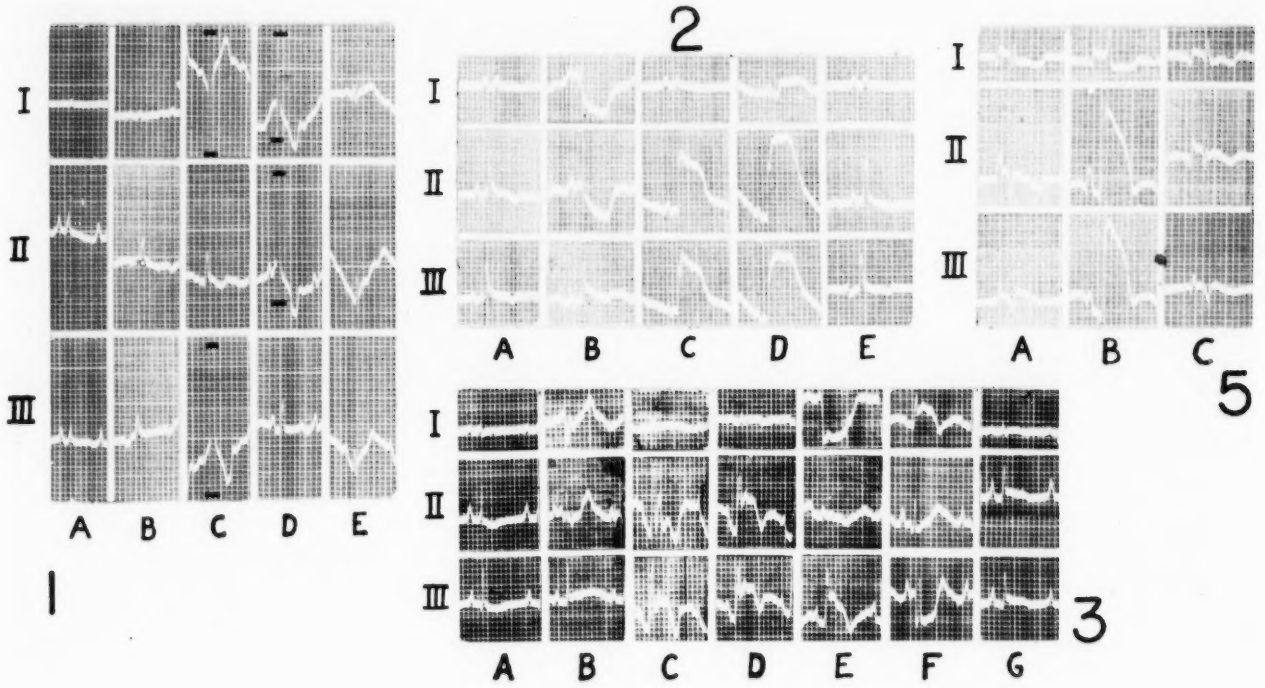
Recently, we have shown (Katz and Korey) that the tissues adjacent to the heart are not all equally good electrical conductors, the lungs and the large systemic vessels being much poorer conductors than the posterior paravertebral muscle mass, the diaphragm, and the chest wall. The introduction of insulators between the heart and the good electrical conductors caused a decided decrease in the amplitude of the deflections recorded in the standard limb leads. On the other hand, separation of the heart from the large systemic vessels, and the insertion of insulators between the heart and lung had an insignificant effect on these deflections. On the basis of the above data, the changes in the electrocardiogram which occur on altering the heart's position in the dog (Katz and Ackerman, Ackerman and Katz) and in man (Meek and Wilson, Nathanson, Katz and Robinow) have been interpreted as being the result of 1, changes in the regions of the heart in contact with the good conductors, and 2, changes in the position of the good conductors in the chest. It was deemed desirable to put this concept to further experimental test. In the present study this was done by noting whether or not the introduction of good electrical conductors between various regions of the heart and various parts of the body would definitely modify the electrocardiogram obtained from the usual limb leads. Some preliminary experiments of Smith and Kountz seemed to indicate that this was possible. They found that the ordinary three lead electrocardiogram obtained from a human cadaver, after connecting to it the heart of an isolated dog heart-lung preparation by means of an electrical shunt, was modified by changing either the location of the contact on the chest of the cadaver or that of the contact on the dog's heart. In the present investigation was noted the effect on the three lead electrocardiogram of shifting the chest contact of a metallic shunt which was in contact with a fixed region of the heart in the open chested anesthetized dog.

[1] A preliminary report was presented to the American Physiology Society, March, 1935.

METHOD. Twenty-three dogs anesthetized with sodium barbital were used in this study. The chest was opened anteriorly after artificial respiration was induced. The heart was maintained constant in position by means of a cradle formed of the opened pericardial sac. The usual German silver electrodes wrapped in saline soaked gauze were inserted subcutaneously in the three extremities used to obtain the standard electrocardiograms. At first, cotton yarn 2 mm. in diameter and about 8 or 10 cm. in length and soaked in saline agar was used for the heart contact. This was connected through an ordinary non-polarizable boot electrode to copper wire. The latter was connected in turn to various parts of the chest by means of a selector switch.

The cotton yarn contact was soon discarded as having too great an electrical resistance, and either a boot electrode wrapped in saline-soaked gauze was applied directly to the heart, or instead a camel's hair brush impregnated with 0.9 per cent NaCl kaolin. In the latter case, the hollow handle was filled with copper sulphate and the wire inserted into this solution. Control curves with the heart contact in place but with the shunt unconnected were obtained before and after each experiment. In some experiments, the heart was permitted to remain in contact with the posterior paravertebral muscle mass. In many, however, the heart was lifted away in order to decrease the magnitude of the electrocardiographic deflections obtained in the controls. In other experiments a rubber sheet was inserted beneath the heart for the same purpose. Usually the anterior surface of the ventricles was used for the heart contact, occasionally the lateral, and rarely the posterior surface was used.

DISCUSSION OF RESULTS. It was soon apparent that two types of alterations in the electrocardiograms were produced by shunting the action currents from the heart directly to the chest. These may be briefly described as *a*, a diphasic addition (consisting of a diphasic QRS complex with a T wave opposite in direction to the last phase of QRS), and *b*, a monophasic addition (consisting of an S-T deviation with a T wave, when present, in the opposite direction). At times combinations of the two types of additions were seen. In figure 1 is shown one of the most striking diphasic additions and in figure 2 a marked monophasic addition. It was soon found that the monophasic addition was due to the production of injury beneath the shunting electrode on the heart. The ease with which injury currents are set up accord with previous observations from this laboratory (Jochim, Katz and Mayne). In several experiments, successive records revealed the nature of the transition from the diphasic to the monophasic addition (cf. fig. 3). The first stage in such a transition consisted in a splintering of the QRS complex, an increase in its time span, and a notching of the T wave (viz., segments B and C). Later, S-T deviations with T waves in the opposite direction appeared; the S-T deviation then be-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came more marked, and the T wave decreased in size until, ultimately, a more or less monophasic curve developed. It would appear that the first stages were produced by marked asynchronism of the events beneath the shunt electrode on the heart. This we have ascribed to a slowing of the conduction of the impulse within the region beneath the shunt electrode, a stage apparently occurring before the muscle tissue is injured sufficiently to give rise to the injury currents.

It is obvious from these results that certain regions of the heart may gain a decided advantage over the rest of the heart in affecting the electrical field merely because they are in contact with a good electrical conductor, with the result that the events in the regions beneath the shunt electrode dominate the electrocardiogram in the standard limb leads. If no injury of the heart tissues is produced beneath the shunt electrode, the addition in the electrocardiogram is a diphasic one. If very slight injury is present in this region, the diphasic addition has a prolonged initial deflection resembling the curve seen in intraventricular block. If more injury is induced in

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Fig. 1. Standard three lead electrocardiogram illustrating the diphasic addition obtained in an open chested dog with the heart in a pericardial apron raised to separate it from the posterior paravertebral muscle mass. Segment A, control; B, after boot electrode was placed on left ventricle anteriorly but not connected with any chest spot; C, shunt connected to left axilla (the black lines indicate the height of the diphasic QRS which in lead I has its 1st phase up and 2nd down, and in lead III has its 1st phase down and 2nd up); D, shunt connected to right axilla (the black lines indicate height of diphasic QRS which in leads I and II has its 1st phase down and 2nd up); E, shunt connected to left chest wall laterally at level of 5th rib (in all three leads a small diphasic QRS is present with 1st phase up and T is upright).

Fig. 2. Standard three lead electrocardiograms illustrating the monophasic addition obtained in an open chested dog with the heart in contact with the posterior paravertebral muscle mass. Segments A and E, control with boot electrode placed on left ventricle anteriorly but not connected with any chest spot; B, C, and D, shunt connected respectively to right axilla, to right lateral chest wall at level of 5th rib, and to left lateral chest wall at level of 5th rib.

Fig. 3. Standard three lead electrocardiograms illustrating the gradual development of the monophasic addition obtained in an open chested dog with the heart in contact with posterior paravetebral muscle mass. Discussed in text. Segment A, control before boot electrode applied. Segment G, control with boot electrode for shunt placed on left ventricle anteriorly but not connected with any chest spot. Segments B, C, D, E, and F, shunt connected respectively with right lateral chest wall at the level of second rib, with the same at the level of fifth rib, with the middle of the diaphragm, with the left lateral chest wall at the level of the 5th rib, and the same at the level of the second rib.

Fig. 5. Standard three lead electrocardiograms obtained in an open chested dog illustrating the appearance of a monophasic addition following the placing of the injured posterior wall of the ventricle in contact with the posterior paravertebral muscle mass. Injury was produced by a boot electrode. In segments A and C the heart apex was elevated to separate the heart from the posterior paravertebral mass; in segment B, the heart is in contact with this muscle mass. Discussed in text.

this region, the addition is a typical monophasic one, resembling the type of record seen following extensive injuries to the heart.

While some disturbance in the electrocardiogram is produced by the placement of the contact electrode on the heart (either by producing injury, by altering the heart shape, or by offering a shunt from one region of the heart to another), this is relatively insignificant (cf. figs. 1A and B and figs. 3A and G).

A rough estimate of the data in the various experiments indicates that the magnitude of the addition depends on the electrical resistance of the shunt employed. The magnitude of the addition in the electrocardiogram as well as its direction is modified also by the location of the point on the chest to which the shunt electrode is connected. It appears that the shunt sets up its own electrical field in the body, and the results in each lead depend on the relative proximity of the two recording electrodes to this spot. Since the electrical resistance of the tissues in the body conveying these electrical currents is not the same, one would not expect an exact parallelism between the ratio of the linear distances of the recording electrodes from the shunt spot on the chest and the direction and magnitude of the addition in the electrocardiogram. Our results show, however, a rough parallelism (cf. figs. 1, 2 and 3).

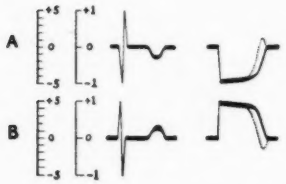

Fig. 4. Diagram showing the appearance of the diphasic (on the left) and monophasic additions (on the right). The diagram indicates by the two scales that variations in magnitude of the additions can be obtained. The opposite directions which the diphasic and monophasic additions may take, depending on the location of the shunt point on the chest to the recording electrodes is indicated by curves A and B. The dotted line in the monophasic additions shows its appearance when less injury is present than that required to give the solid line curves. Discussed in text.

It is possible that the relative "electrical distance"[2] of the two recording electrodes from various shunt spots on the chest can be calculated in each lead by the magnitude and direction of the addition. Thus, when the two recording electrodes are equidistant "electrically," no addition is obtained since the effects on the recording electrodes neutralize each other (viz. lead II, segment C and lead III, segment D of fig. 1; also, lead III, segment B and lead I, segment C of fig. 2). When, however, the "electrical distance" of one recording electrode from the shunt spot is much less than that of the other, a large addition is obtained (viz., leads I and III of segment C, and leads I and II of segment D of fig. 1; leads I and II of segment B, and leads II and III of segments C and D of fig. 2). Smaller

[2] By "electrical distance" we mean the linear distance multiplied by the mean specific electrical resistance of the tissues between the two points considered.

additions are obtained when there is less disparity in the "electrical distance" of the two recording electrodes to the shunt point (cf. segment E of fig. 1, and lead I of segment D of fig. 2).

It was found that with the conventional connections used, the diphasic addition consisted of a diphasic QRS with the 1st phase inverted, and an inverted T wave, and the monophasic addition consisted of a depressed S-T segment (and upright T wave) when the chest contact of the shunt was nearer to the right arm electrode of lead I and II and the left arm electrode of lead III (cf. fig. 4A). The inverse image was obtained when the chest contact of shunt was nearer to the other electrode of the above leads (cf. fig. 4B). It is obvious that the electrical field created by the shunt can be mapped out from such data.

The importance of good electrical contact in giving particular regions of the heart an advantage is shown also by an experiment illustrated in figure 5. In this dog the heart had been separated from the posterior paravertebral muscle mass so as to permit the application of a boot electrode on the posterior surface of the heart for shunting purposes. This boot had produced injury beneath it. When segment A was taken, the boot had been removed, and the usual type of electrocardiogram was obtained such as has been described by Katz and Ackerman. When, however, the heart was placed back in contact with the posterior paravertebral muscle mass, a marked monophasic addition was obtained in leads II and III and none in lead I (viz., segment B). This addition disappeared when the heart was returned to its preceding position (viz., segment C). It is obvious that the injury had persisted after the boot was removed, but did not affect the electrocardiograms because it was not advantageously situated. By placing this injured area in contact with the posterior paravertebral muscle mass, it gained sufficient advantage to dominate the curve. Furthermore, the absence of a monophasic deflection in lead I indicates that the point of contact on the posterior paravertebral muscle mass was approximately "electrically equidistant" from the two forelimb electrodes, while the presence of large upright monophasic curves in leads II and III, indicate that the point of contact on the posterior paravertebral muscle mass was electrically closer to the hindlimb than to the forelimb electrodes. It is difficult to see how the usual vector analysis and the Einthoven equilateral triangle concept could explain this observation.

SUMMARY

1. Experiments are reported in which various regions of the heart were connected to various parts of the chest by a good electrical non-polarizable shunt. The alterations obtained were of two sorts (or intermediate or mixed forms): *a*, diphasic additions when the region under the shunt elec-

trode was not injured, and *b*, monophasic additions when the region under the shunt electrode was injured.

2. It is demonstrated that regions of the heart may gain a decided advantage over the rest of the heart merely because they are in contact with a good electrical conductor, provided the contact is electrically nearer to one than the other of the two recording electrodes.

3. It is demonstrated that the point on the chest to which the heart currents are shunted determines the advantage of the heart region so shunted over the rest of the heart. This depends on the "electrical distances" between the recording electrodes and the point of shunt on the chest.

4. It is demonstrated that the electrical resistance of the shunting circuit determines the degree of advantage gained by the heart region shunted; the less the electrical resistance of the shunt, the greater the advantage.

5. It is suggested that the shunted region of the heart sets up its own electrical field in the body which summates with that set up by the heart through its natural contacts.

6. These experiments support the concept that the nature of the electrical conductors in contact with the heart are an important, if not the most important, element in determining the nature of the electrical field set up by the heart and thereby in determining the contour of the electrocardiogram obtained with recording electrodes at a distance from the heart. They also support the concept that alterations in the relation of various regions of the heart to the good electrical conductors and alterations in the location of the latter are important factors in modifying the ordinary electrocardiogram.

REFERENCES

ACKERMAN, W. AND L. N. KATZ. Amer. Heart, J. **8:** 490, 1933.
JOCHIM, K., L. N. KATZ AND W. MAYNE. This Journal **111:** 177, 1935.
KATZ, L. N. AND W. ACKERMAN. J. Clin. Invest. **11:** 1221, 1932.
KATZ, L. N. AND H. KOREY. This Journal **111:** 83, 1935.
KATZ, L. N. AND M. ROBINOW. Amer. J. Med. Sci. (in press).
MEEK, W. J. AND A. WILSON. Arch. Int. Med. **36:** 614, 1925.
NATHANSON, M. H. Proc. Soc. Exper. Biol. and Med. **28:** 766, 1931.
SMITH, J. R. AND W. B. KOUNTZ. J. A. M. A. **104:** 148, 1935.

BLOOD SUGAR AND GLUCOSE TOLERANCE AT HIGH ALTITUDES[1]

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The literature dealing with the effect of low oxygen tensions upon the sugar in the blood is not very extensive, but it presents two points of view: that lowering the oxygen tension has no effect upon the level of the blood sugar (1), (2), and that it raises it (3), (4), (5), (6). These findings are less contradictory than they at first appear, for in general the authors who have reported no change have worked on better acclimatized subjects and at lower altitudes than those who report increases. Anoxemia raises the blood sugar in cats even in the absence of excitement (7), but reducing the pressure to 340 mm. does not increase the blood sugar in guinea pigs (8). In the anoxemia of CO poisoning the amount of sugar in the blood has been found to increase (9), (10). But in recent unpublished experiments the author found no change in the blood sugar in men even when the blood became as much as 45 per cent saturated with CO. When the oxygen tension is greater than normal, the blood sugar either stays the same (11) or decreases (12).

During the recent months in the Chilean Andes we were able to extend previous observations and to follow the blood sugar in 10 healthy subjects both in rest and work as they became acclimatized to various heights up to 5,340 meters and to get a few values in rest from 6,140 meters. We also made four sugar tolerance tests at 5,340 meters, described in the last part of this paper.

Care must be exercised in choosing a method for sugar determinations at altitudes over 2,000 meters because the lowering of the boiling point

[1] From the International High Altitude Expedition to Chile. The Expedition was supported by grants from the Fatigue Laboratory and the Milton Fund, Harvard; Duke University; Copenhagen University; King's College, Cambridge University; Columbia University; the National Research Council; the Royal Society, London; the Corn Industries Research Foundation; the Rask-Ørsted Fund, Copenhagen; the Josiah Macy Foundation; and the American Association for the Advancement of Science. We are also much indebted to the Chile Exploration Company and to its officers in New York and in Chile, to Señores Hipolito and Juan Carrasco of the S.I.A.M. Carrasco, to Messrs. Packard and Bell of the Poderosa Mining Company, to the Ferro-Carril de Antofagasta a Bolivia, and to the many people, both officials and private citizens, who facilitated our work in Chile.

of water affects all ordinary methods to a certain extent and renders some quite unreliable (13), (14). The revised Folin-Wu method (15) is one which is but little affected and was chosen for this reason and for its convenience. Filtrates were prepared both by the usual Folin-Wu precipitation (16), which leaves in the filtrate the glutathione and other reducing substances coming from the cells, and by the "unlaked" Folin method (17), which gives filtrates that are relatively free from reducing substances other than glucoss.

It was hoped that the differences between the values obtained with the laked and with the unlaked filtrates would give the amounts of non-glucose reducing substances, but unfortunately the uncertain interplay of three factors made these differences an unreliable index to the amount

TABLE 1

Average values for resting and fasting blood sugars at different altitudes

Two methods were used, and the difference between the values by the two methods is shown. The number of determinations entering into the average figure is given by the superscript, and the number of individuals from whom the samples were taken is given in the last row.

	SEA LEVEL	PLACE				
		Chuqui	Ollagüe	Montt	'Quilcha	Punta*
Altitude in meters	0	2,810	3,660	4,710	5,340	6,140
Sugar in mgm./100 cc. of blood by "laked" method	107^{7}	94^{13}	85^{5}		91^{7}	90^{6}
Sugar in mgm./100 cc. of blood by "unlaked" method	78^{3}	74^{17}	69^{2}	79^{1}	86^{2}	83^{4}
Difference	29	20	16		5	7
Number of individuals	4	10	6	1	4	5

* All samples brought down and determinations made at 'Quilcha.

of these substances. The first of these factors was the effect of altitude upon the method. The time required for the reaction between the sugar and the copper reagent to reach 98 per cent of its final value is about 5½ minutes at sea level, 10 minutes at 2,810 meters (91°C.), and 20 minutes at 5,340 meters (83°C.). This slowing of the reaction increases the "copper number" (13), (14) and makes the reagent more selective with respect to glucose (13), thereby making the non-glucose reducing substances appear less. The second factor is a slight uncertainty as to the completeness of precipitation when the cell volume is very high (the greatest cell volume found in a member of the Expedition was 63.6 per cent, in a native 82.5 per cent). The third factor was the difficulty of making accurate determinations under the conditions which often obtain during an expedition. Many of the resting fasting values were obtained

from arterial blood. They were close to the venous values, as Foster (18) found, and are included with them. All the values in work, in recovery from work, and after the ingestion of 100 grams of glucose, were obtained from venous blood.

The values obtained for the blood sugar in rest at different altitudes in the members of the Expedition are given in table 1. It will be seen that with the "unlaked" method the values remained constant up to 3,660 meters and above that rose slightly. With the "laked" method there was a fall as the altitude increased up to 3,660 meters (due presumably to the increasing selectivity of the method, as explained above, and not to any change in the amount of glucose), and a very slight rise above 3,660. It is uncertain whether these slight rises in blood sugar result from the low oxygen tensions. In general, the subjects were colder and less completely at rest at the higher altitudes than at the lower. Either or both these factors may well account for a slight rise (19), and an analysis of the average values (83 mgm. "unlaked" and 90 mgm. "laked") obtained at 6,140 meters shows how considerable an increase in sugar can be produced by a little activity. Three of the five subjects there were in a thoroughly resting state, and they gave blood sugars by the unlaked method of 72 and 65 mgm., and by the laked of 88,[2] 81, 88, and 79[2] mgm. Two subjects had moved around considerably and only rested for a short while before the blood was drawn; their values were 104 and 91 unlaked, and 108 and 94 laked. On the other hand, the plasma contains more glucose per cubic centimeter than the corpuscles, and the corpuscle volume was larger at the higher altitudes. Consequently, the sugar in the plasma must have increased with altitude more than the sugar in the whole blood. (All values in this paper are for whole blood.) In order to calculate what the plasma sugar was, both the corpuscle volume and the distribution of sugar between the corpuscles and plasma must be known, but upon the latter there is no agreement (20), (21), (22), (23). Taking Svedberg's (22) value of 0.60 for the C/Pl. ratio for glucose, and the average change in corpuscle volume (from 44.3 to 54.3 per cent),[3] it appears that a 10 mgm. increase in the whole blood sugar from 74 to 84 mgm. represents a change in the plasma sugar from 90 to 107 mgm. per 100 cc. of plasma. If Olmstead (23) is correct in thinking there is no sugar in the corpuscles while they are in the body, then the average of the plasma sugars for members of the Expedition must have changed from 132 mgm. to 182, and the natives whose whole blood sugars were around 75 and corpuscle volumes 70 to 80 must have had plasma sugars of the order of 300 mgm. The few experiments I have done on distribution confirm Svedberg rather than Olmstead, so that the change in cor-

[2] Same individual July 7th and July 10th respectively.

[3] These figures were kindly given to me by Dr. J. H. Talbott.

puscle volume does not invalidate the conclusion that the change in sugar concentration is not great and that it is uncertain how far it can be ascribed to the lowered oxygen tensions. All subjects were fairly well acclimatized before the experiments were made and there was no general change with further acclimatization.

Blood sugar in work. The literature of this subject is quite extensive, and will not be discussed here, but there are two papers (24), (25) in which the technique and general conditions were enough like those used on the expedition so that they are suited for comparison. Our method was in

TABLE 2

Average changes in blood sugar in work at various altitudes given in per cent of the value for the blood sugar in the lowest grade of work

The superscripts show the number of determinations entering into the average. The individual variations were not great, the average difference between an observation and the mean of its group being less than 4 mgm.

	SEA LEVEL	ALTITUDE (IN METERS)			
		2,810	3,660	4,710	5,340
Rest before work	+16[4]*		+17[1]		+13[1]
Work in kgm.-m. per minute. Blood taken at end of 10 minutes of work:					
225	0	0	0	0	0
455	+2[2]	−1[6]		−6[1]	−3[4]*
680	0[2]	0[5]	+10[3]		−3[4]*
800				0[4]	
910	−2[2]	−1[6]	+5[6]*	+10[1]	
1,020				+10[4]	
1,130	−8[3]*	−1[6]	+20[3]		
1,360		+17[1]			
Recovery	+24[1]			+28[3]	

* Two methods (laked and unlaked) used on the same blood sample and both values included in the average so that the number of individuals represented is 1 or 2 less than the superscript.

general similar to that in the second paper—the subjects rode on a bicycle ergometer, usually for 10 minutes, and blood was taken before the start and again just as the work was ending, or within 15 seconds of the end. After a rest of 10 to 20 minutes the next higher grade of work was begun and blood taken again at the end of it, but usually no sample was taken during the rest periods until the rest after the final period of work. The work during the first period was usually at the rate of 226 kgm.-m. per minute, the second at double this rate, the third at triple it, and so on. For most of the subjects five times the initial work, or 1,130 kgm.-m.

per minute, was as much as they could do at 3,660 meters or below, while at 5,340 meters they could do no more than 680 kgm.-m. per minute, or three times the initial rate. There were nineteen experiments in all, two of which were at sea level, seven at 2,810 meters, three at 3,660 meters, four at 4,710 meters, and three at 5,340 meters. The data are given in table 2. The results were substantially the same at all altitudes. The blood sugar fell from the resting value during light work, as Christensen (24) found in his experiments. It tended to rise as the work became heavy, as Dill, Edwards and Mead (25) found, and it rose sharply immediately after work, as both these papers report. The rise as the work becomes maximal was not so large as in the "discontinuous" experiment of Dill, Edwards and Mead, but there are several factors which may account for that: in our experiments the work, except in the last period, was not so heavy; the rest periods were much longer; and the subject was riding a bicycle instead of running.

Glucose tolerance. At 5,340 meters three subjects who had gone without breakfast were given 100 grams of glucose dissolved in 700 cc. of water, flavored with a little lemon, and this they drank in the course of five minutes. Blood samples were taken at 30, 90 and 180 minutes after the drinking of the glucose. The results were quite striking: two of the subjects who had been at 5,340 meters (or above) for 17 days showed almost no rise in blood sugar (6 mgm. for one and 2 mgm. for the other), while the subject who had been at 5,340 for only 6 days showed a rise of 38 mgm. The experiment was repeated on one of the first two subjects with sucrose with the same result, a rise of only 4 mgm. There was no significant change in the reducing substances in the urine in any subject. The same subjects who showed almost no rise at 5,340 meters were tested again one week after they reached sea level and showed rises in blood sugar of 38 and 52 mgm. respectively. The tolerance curves for these two subjects are shown in figure 1A. The third subject, who after 6 days at 5,340 meters gave a normal curve, had abnormal curves at sea level, figure 1B. Three months later the same experiment was performed in a chamber with a normal barometric pressure but only 12 per cent O_2. The glucose was taken one hour after entering the chamber. The results, given in figure 1C, are on the border of the normal range.

It is difficult to interpret the great increase in glucose tolerance in two of the subjects. (In this paper "tolerance" refers to the sugar in the blood after the ingestion of glucose and not to the sugar in the urine.) They had been losing weight rather steadily (as had all members of the party), one of them had a large appetite and was eating rather heavily, the other's appetite was a little subnormal. In both of them the rectal temperature at this altitude was about 0.6°C. above what was normal for them at sea level. The loss of weight suggests undernourishment, but it has been

shown that starvation decreases glucose tolerance (26), (27) and that diets low in carbohydrates and high in protein or fat decrease the tolerance (27), (28), (but see (29)). The extensive literature has been sum-

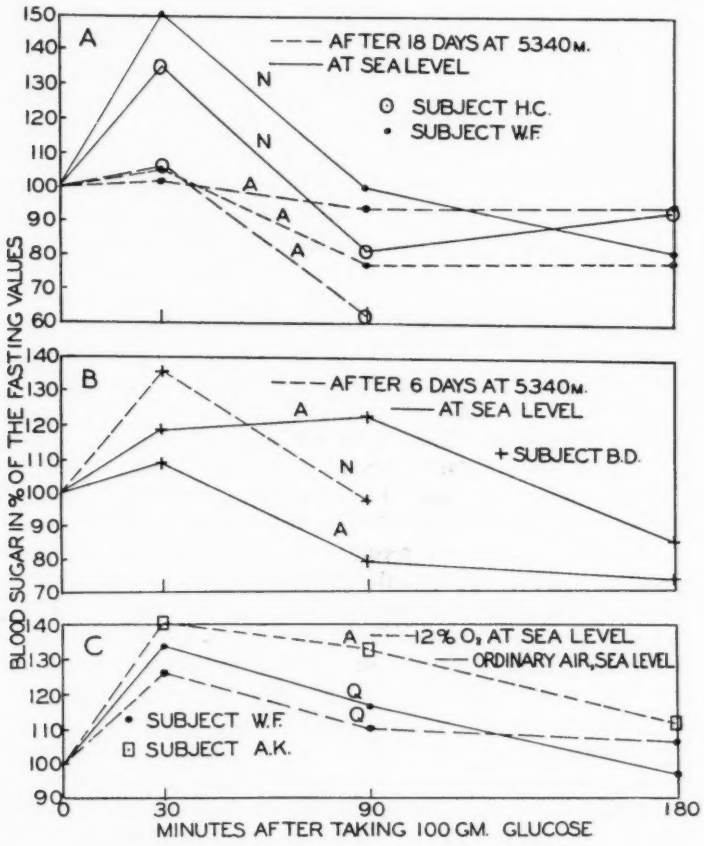

Fig. 1. Glucose tolerance curves. A, at sea level, and after 18 days at 5,340 meters. B, another subject, at sea level, and after 6 days at 5,340 meters. C, in a chamber with 12 per cent O_2 for 1 hour before taking the glucose and during the test, and in ordinary air. The curves marked *N* are clearly within the normal range, those marked *Q* are on the border of it, and those marked *A* are, to different degrees, abnormal.

marized by various authors (26), (27), (29), (30), but they mention only two circumstances (outside of pathology) in which the tolerance is increased, namely, after repeated doses of glucose, and in children after

ketosis has been abolished by a normally balanced diet. Neither of these conditions obtained in our experiments, and it is hard to see what could cause the increase. The main pathological conditions in which glucose tolerance is often high are thyroid deficiency, pituitary deficiency, Addison's disease and scleroderma. There are two papers reporting unchanged glucose tolerance at low oxygen tensions, one in rabbits (31), and one in men (5).

Evans (32) working on rats gives figures showing a small increase in the blood sugar and a very large increase in liver glycogen when they were kept at $\frac{1}{2}$ atmosphere for 24 hours. This suggests a possible explanation for the increased glucose tolerance, namely, a low glycogen content at the time of the tests due to a slightly insufficient diet over a period of weeks (cf., the loss of weight) and the 15 hours' fast immediately preceding the tests, combined with a tendency of the liver to contain (and therefore presumably to form) more glycogen at low O_2 pressures. However, Wertheimer (8) and Montuori and Sapegno (30) state that there is less glycogen in the livers and also in the muscles of mice, guinea pigs, and dogs under diminished pressures.

SUMMARY

Determinations were made of the amount of sugar in the blood of acclimatized subjects in rest and work at various altitudes up to 6,140 meters. Glucose tolerance tests were given at 5,340 meters.

The blood sugar in rest rose slightly with altitude, but it is not certain whether this can be attributed to the altitude.

The behavior of the blood sugar in work was approximately the same at all altitudes.

The glucose tolerance was greatly increased at 5,340 meters in two subjects and changed, though not increased, in a third who has an abnormal tolerance curve at sea level.

REFERENCES

(1) Abderhalden, E., N. Kotschneff et al. Pflüger's Arch. **216:** 362, 1927.
(2) Frenkel-Tissot, H. C. Deutsch. Arch. klin. Med. **133:** 286, 1930.
(3) Baičenko, I. P. and A. N. Krestownikoff. Arbeitsphysiol. **6:** 359, 1932.
(4) Madon, F. Arch. sci. biol. (Italy) **17:** 41, 1932.
(5) Ferraloro, G. Arch. sci. biol. (Italy) **13:** 109, 1929.
(6) Goldberger, S. Boll. soc. ital. sper. **4:** 710, 1929.
(7) Kellaway, C. H. J. Physiol. **53:** 211, 1919.
(8) Wertheimer, E. Ztschr. ges. exper. Med. **70:** 309, 1930.
(9) Lewin, L. Kohlenoxydvergiftung, p. 207 et seq. Springer, Berlin, 1920.
(10) Mikami, S. Tohoku J. Exper. Med. **8:** 237, 1927.
(11) Shilling, C. W., R. M. Thompson, A. R. Behnke, L. A. Shaw and A. C. Messer. This Journal **107:** 29, 1934.
(12) Aggazzotti, A. and A. De Niederhaüsern. Boll. soc. ital. sper. **7:** 1255, 1932.

(13) FORBES, W. H. AND A. ANDREEN-SVEDBERG. Skand. Arch. Physiol. **70:** 168, 1934.
(14) WOOD, W. B., JR. J. Biol. Chem. **110:** 219, 1935.
(15) FOLIN, O. J. Biol. Chem. **82:** 83, 1929.
(16) FOLIN, O. AND H. WU. J. Biol. Chem. **38:** 81, 1919.
(17) FOLIN, O. J. Biol. Chem. **86:** 173, 1930.
(18) FOSTER, G. L. J. Biol. Chem. **55:** 291, 1923.
(19) KRAMER, B. AND H. W. COFFIN. J. Biol. Chem. **25:** 423, 1916.
(20) FOLIN, O. AND A. SVEDBERG. J. Biol. Chem. **88:** 715, 1931.
(21) SOMOGYI, M. J. Biol. Chem. **90:** 731, 1930.
(22) ANDREEN-SVEDBERG, A. Skand. Arch. Physiol. **66:** 113, 1933.
(23) OLMSTEAD, J. M. D. This Journal **114:** 488, 1936.
(24) CHRISTENSEN, E. H. Arbeitsphysiol. **4:** 128, 1931.
(25) DILL, D. B., H. T. EDWARDS AND S. MEAD. This Journal **111:** 21, 1935.
(26) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. 1, p. 122. Williams and Wilkins, Baltimore, 1931.
(27) HIMSWORTH, H. P. J. Physiol. **81:** 29, 1934.
(28) SWEENEY, J. S. Arch. Int. Med. **40:** 818, 1927.
(29) JOHN, H. J. J. Metab. Res. **1:** 497, 1922.
(30) MONTUORI, S. AND E. SAPEGNO. Arch. soc. biol. (Italy) **20:** 286, 1934.
(31) MONASTERIO, G. Ztschr. ges. exper. Med. **70:** 314, 1930.
(32) EVANS, G. This Journal **110:** 273, 1934; **114:** 297, 1936.

THE INFLUENCE OF BILE ON THE EXCRETION OF STEROL IN THE FECES

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The relation of bile to the excretion of sterols in the feces of dogs has been extensively studied, but the nature of the observations hitherto made on humans has been such that definite conclusions could not safely be drawn from them. Sperry (1) showed that on a lipid free diet, dogs with complete bile fistulas excreted from 1.5 to 4.5 times as much unsaponifiable material in their feces as in the normal state. On the basis of colorimetric analyses, he concluded that "cholesterol" excretion was also increased in the same proportion. Beumer and Hepner (2) found that in the feces of dogs fed on a diet containing 19 mgm. of sterol per day, the sterols estimated as cholesterol by digitonin precipitation increased to from 4 to 7 times the normal level after a bile fistula was established. On a cholesterol free diet their dog excreted 0.026 gram of sterol per day. This rose to 0.136 gram per day after a bile fistula had been established and fell to 0.085 gram per day when 100 cc. of bile containing 0.014 gram of cholesterol was added to the diet. No exactly comparable studies have been made in humans. Beumer (3) found that a patient with complete common bile duct obstruction excreted slightly less sterol than a patient without organic disease on the same diet. Salomon (4) also found that a patient with complete bile obstruction excreted essentially the same amount of sterol as normal controls.

This report deals with observations on two patients whose bile could be diverted from or let into their intestinal tracts at will for intervals of several weeks. Both patients were operated on because of recurrent biliary obstruction following cholecystectomy. At operation, the bile duct was found to be greatly dilated and a wide rubber T-tube was inserted for drainage. In each case, as long as the tube was left open, the resistance of the sphincter at the ampulla of Vater was sufficient to keep the stools free from bile pigment. When the external end of the T-tube was closed, the bile ran into the intestines and bile pigment appeared in the stools within twenty-four hours. Further details about the fistulae, which govern the validity of subsequent observations, are completely described in the case of D. W. in a previous paper (6).

Diet. The patients were on the ordinary hospital low-fat diet. A careful record of everything eaten by D. W. during period B was kept. The diet in her subsequent periods followed this as closely as possible. In period D her diet was weighed and a duplicate was analyzed by the same procedure as that used for the stools. In the case of patient H. the diet was one-half by weight of that eaten by D. W. during period D. The stools were demarcated by 0.5 gram of carmine given at the beginning and end of each period.

The total stools for each period were collected, mixed thoroughly, acidified with glacial acetic acid and dried by powdering with anhydrous sodium sulphate. About 500 grams of powdered stool were continuously extracted with hot ether for 24 hours in a modified Clark extractor[1] (5). Subsequent 24 hours' extraction yielded only traces of ether-soluble material. The ether extract was washed free of Na_2SO_4 with water and evaporated to constant weight on a steam bath in a stream of air. After saponification by refluxing for two hours on a steam bath with 7 per cent KOH in 90 per cent methyl alcohol, the contents of the flask were washed into a separatory funnel with ether and water. More ether and water were added and the contents shaken gently to extract the ether soluble material. After standing long enough to give a sharp separation of the two layers, the water was drawn off into a second separatory funnel and repeatedly shaken with ether until the ether layer was practically colorless. The combined ether layers were washed once with water and this water layer extracted again with ether. All ether layers were combined and washed repeatedly with water until free of alkali. The ether solution containing the unsaponifiable fraction was then evaporated to constant weight on a steam bath in a stream of air. The sterols in the unsaponifiable fraction were determined gravimetrically after precipitation by digitonin in 80 per cent ethyl alcohol. Most of the procedures were done in duplicate and checked within a few percent.

The results are given in table 1. It is clear from the table that in the absence of bile from the intestines the total weight of ether soluble material excreted in the feces was about 3 to 4 times the normal amount. The excretion of unsaponifiable material however remained practically unchanged in the case of D. W. and increased to about 1.5 times the normal in case H. The excretion of digitonin precipitable sterols in both cases was definitely less than when bile was present, even though the difference between two bile free periods is greater than the difference between one bile free period (D) and one period with bile (C). In all periods more sterols were excreted than were ingested.

The increase in the total ether soluble material of the feces in the periods when the fistula was open is in agreement with the findings in practically all similar studies on humans or animals without bile in their intestinal tract. It is of interest here, principally, as additional confirmation of the exclusion of bile from the intestines.

In the excretion of sterols, however, our patients reacted to the exclu-

[1] A coil of $\frac{1}{4}$ inch copper tubing was used instead of a flask as a condenser.

TABLE 1

Fecal sterols with and without bile fistula

PATIENT	PERIOD	TIME	PROCEDURE AND CONDITION OF EXPERIMENT	WEIGHT OF ETHER EXTRACT OF FECES PER DAY	WEIGHT OF UNSAPONIFIABLE MATERIAL PER DAY		STEROLS PER DAY	
					Diet	Feces	Diet	Feces
				grams	grams	grams	grams	grams
D. W.		1/5/35	Operation: Stones removed from common and hepatic ducts. T-tube inserted in common duct. Free bile drainage. No bile in stools					
		2/17	Drainage tube closed. Patient ambulatory. Bile running freely into intestines. Bile pigment in feces shortly after					
	B	2/25 to 3/7 (10)	Bile fistula closed. Ambulatory. Bile running freely into intestines. Bile pigment in feces	2.93	0.86	1.35	0.115	0.755
	C	3/26 to 4/5 (10)	Same as above	3.91	0.86	1.16	0.115	0.729
		4/6	Tube opened. Bile allowed to run out through drainage tube					
	D	4/13 to 4/20 (6)	Bile fistula open. Patient in bed. No bile pigment in feces. Total of 4.2 gm. of extra fat like olive oil by mouth on two middle days	9.90	0.87	1.18	0.119	0.518
	E	5/9 to 5/20 (10)	Bile fistula open. Ambulatory. No bile pigment in feces	10.3	0.86	0.90	0.115	0.235
H		6/10	Operation: common duct reconstruction. T-tube inserted. Free bile drainage. No bile in stool					
	H_1	6/30 to 7/5 (5)	Bile fistula open. Patient in bed. No bile pigment in feces; 10.5 gm. extra fat (olive oil) by mouth on middle day	6.90	0.43	0.78	0.087	0.197
		7/7	Tube closed. Patient in bed					
	H_2	7/18 to 7/23 (5)	Bile fistula closed. Patient in bed. Bile running freely into intestines. Bile pigment in feces	1.43	0.47	0.47	0.058	0.274

sion of bile in a manner different from that of the dogs of Sperry (1) and of Beumer and Hepner (2). Sperry believed that the amount of sterol in the feces was maximal in bile fistula subjects because the absence of bile salts hindered the reabsorption of sterols excreted by the intestinal mucosa. Though our data are in complete agreement with his findings, that sterols are excreted by the intestinal mucosa, the fact that our patients excreted larger amounts of sterol in the feces when bile was admitted to their intestines, casts doubt on the applicability to humans of his interpretation of the rôle which bile plays in the reabsorption of sterol.

On the other hand, the presence of relatively large amounts of cholesterol in human bile (7) (8) as compared with dog bile may account for this apparent difference. In the case of H. the difference between the fecal sterols in the normal control and bile fistula periods was 77 mgm. per day. Fox (8) has calculated from data in the literature that human fistula bile, which probably contains no more sterol than normal human liver bile, contains on the average 340 to 400 mgm. of cholesterol per day. If we subtract this from the 274 mgm. of fecal sterol in the period when bile was allowed to run into the intestine, we find that more than the entire amount of sterol secreted by the intestinal mucosa in the bile-free period could have been reabsorbed in the presence of bile. Similar calculations for D. W. show that somewhat larger amounts of cholesterol such as have occasionally been reported (8) for the daily excretion from a bile fistula could easily have changed the apparently increased excretion of fecal sterol when bile was present, to values compatible with those found by Sperry and Beumer and Hepner in dogs. Similar calculations on their data indicate that if the sterol content of dogs' bile had been similar to that of human bile, their results would have been in good agreement with our findings in human subjects.

The above calculations are based on the assumption that the biliary cholesterol may be considered simply as cholesterol added to the intestinal content without any special ease of absorbability. If this assumption is even approximately correct, it may be that the rôle of bile in the absorption of sterols is the same in humans as in dogs. On this basis the apparent difference would be due to the high cholesterol content of human bile.

SUMMARY

Data on human subjects have been presented indicating that fecal sterol excretion is decreased when bile is excluded from the intestines. It is suggested that the apparent difference between our findings in humans and the findings of other observers in dogs may be due to the high cholesterol content of human bile as compared with dog bile.

Grateful acknowledgment is made to Dr. Rudolph Schonheimer for his valuable advice in the course of this work.

REFERENCES

(1) Sperry, W. M. J. Biol. Chem. **71:** 351, 1927.
(2) Beumer, H. and F. Hepner. Ztschr. f. d. ges. exper. Med. **64:** 787, 1929.
(3) Beumer, H. Ztschr. f. d. ges. exper. Med. **35:** 328, 1923.
(4) Salomon, H. Arch. f. Verdauungskr. **41:** 257, 1927.
(5) Sperry, W. M. J. Biol. Chem. **68:** 357, 1926.
(6) Koster, H., A. Shapiro and H. Lerner. This Journal **115:** 23, 1936.
(7) Wright, A. and G. H. Whipple. J. Exper. Med. **59:** 411, 1934.
(8) Fox, F. W. Quart. J. Med. **21:** 107, 1927.

THE SITE AND MECHANISM OF THE ANTIKETOGENIC ACTION OF INSULIN[1]

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The administration of insulin to the depancreatized dog (1) or to the patient in diabetic coma (2) results in an immediate decrease in the acetone body content of the blood. This antiketogenic effect of insulin generally is ascribed to the stimulation of carbohydrate oxidation in the tissues on the assumption that in these conditions there is a decrease in carbohydrate utilization by the muscles. However, there is ample evidence that the depancreatized animal uses carbohydrate in the extrahepatic tissues (3) (4). This fact makes it improbable that the current hypothesis can serve as an adequate explanation of the action of insulin in the prevention of ketosis.

In a previous report we presented evidence which demonstrated that the administration of a neutralized alkaline extract of the anterior lobe of the hypophysis to normal rabbits results in the rapid appearance and accumulation of acetone bodies in the blood. Since this ketosis does not occur in the liverless rabbit, it was concluded that the liver is the sole source of the acetone bodies thus produced (5). In view of these considerations it became of interest to investigate the effect of insulin on the ketogenic action of this anterior hypophyseal extract. If the action of insulin is due only to stimulation of the carbohydrate oxidation in the extrahepatic tissues, it should exert no effect on the formation of ketones by the liver. On the other hand, since insulin has no effect on the rate of the removal of acetone bodies from the blood (6), any inhibitory effect of insulin on the ketogenic action of the anterior hypophyseal extract must be due to some specific action in the liver.

METHODS. This study was carried out on white, male, New Zealand rabbits weighing approximately 2 kgm. All the animals were fasted for twenty-four hours. Sodium pentobarbital anesthesia was employed throughout all experiments to permit the withdrawal of blood samples from the exposed femoral artery. As in the previous study, the "total

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[2] Presented before the American Physiological Society, March, 1936.

acetone" of the blood was determined by a modification of the Van Slyke and Fitz method. Blood samples were drawn before, and at hourly intervals after the administration of the extract. All values below 1 mgm. per cent were regarded as zero since that is within the error of the method.

The neutralized alkaline anterior hypophyseal extract employed in this study was exactly the same as that used in our previous experiments.[3] The administration of the equivalent of two grams of fresh gland was followed immediately by the subcutaneous injection of insulin into the opposite side of the body in doses varying from 1.3 to 3 units per kilogram of body weight.

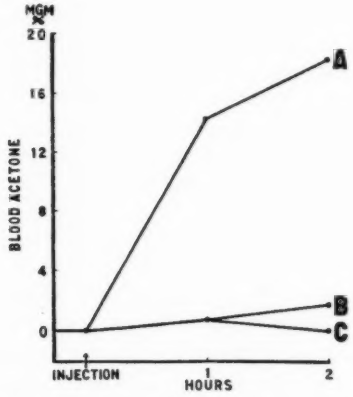

Fig. 1. Graphic representation of the average "total acetone" content of the blood of normal rabbits following the administration of *A*, neutralized alkaline extract of the anterior hypophysis alone; *B*, extract and insulin; *C*, extract and ergotamine.

As a result of our findings in the above experiments, another set of rabbits was treated with the anterior hypophyseal extract after the preliminary intramuscular administration of 2.0 mgm. ergotamine tartrate per kilogram of body weight.

RESULTS. The results are graphically summarized in figure 1. It is apparent that the concomitant administration of insulin produces a marked inhibition of the ketogenic effect of the anterior hypophyseal extract. Whereas the average "total acetone" in the blood of six rabbits treated with the extract alone reaches a value of 14 mgm. per cent in one hour, and 18 mgm. per cent in two hours (fig. 1, *A*), that of the six rabbits

[3] We are indebted to Dr. E. D. Campbell of the Eli Lilly Research Laboratories for generous supplies of powdered, anterior hypophyseal extract and insulin, and to the Sandoz Chemical Works for ergotamine tartrate.

treated with the same extract and insulin is 0.7 mgm. per cent and 1.7 mgm. per cent at the same intervals of time (fig. 1, *B*).

It also may be noted that the preliminary administration of ergotamine tartrate prevents the accumulation of ketones in the blood of rabbits receiving the anterior hypophyseal extract (fig. 1, *C*).

DISCUSSION. These results demonstrate the fact that insulin inhibits the ketogenic action of an extract of the anterior hypophysis which has been shown previously to be effective only in the presence of the liver. In view of this, it becomes probable that the antiketogenic action of insulin must be due to some specific effect on the liver. It is improbable that an acceleration of the removal of glucose from the blood by the extrahepatic tissues is a direct factor in this ketolytic action of insulin, because these tissues do not play any significant rôle in the production of ketone bodies (5). Nor is it likely that the administration of insulin increases the rate of acetone utilization by the tissues since Chaikoff and Soskin have shown that the utilization of this substance is the same in both normal and depancreatized animals (6).

Since insulin is antiketogenic because of some effect on the liver, it becomes of interest to inquire into the mechanism of this action. One function of insulin that is generally accepted is its ability to inhibit the hepatic glycogenolysis induced by various means. This action is indicated by the fact that the liver of the depancreatized dog can make glycogen when glucose is administered in large amounts (7), but cannot retain it unless insulin is administered at the same time. Similarly, insulin inhibits the hepatic glycogenolytic action of epinephrin (8). When this function of insulin is correlated with the fact that the production of ketone bodies is usually found in association with a glycogen poor liver, it becomes probable that the antiglycogenolytic action of insulin may be responsible for its antiketogenic effect. This probability is supported by the fact that insulin exerts a ketogenic action when it is administered to normal adult animals where it is known to produce a depletion of liver glycogen. Thus, Burn and Ling (10) demonstrated that when insulin augmented the winter ketosis of rats fed high fat diets, it concomitantly prevented the accumulation of liver glycogen which normally occurred in these animals. In 1923 Collip (11) observed that the urine collected immediately after the onset of insulin hypoglycemic convulsions in rabbits contained appreciable amounts of ketone bodies. Low and Krema (12) found that insulin always produces a primary rise in the blood and urinary ketone bodies of human subjects and rats. With the latter species they demonstrated that this effect of insulin is associated with an increase in liver ketones and depletion of liver glycogen. It is also noteworthy that ketosis occasionally occurs in association with the hypoglycemia that characterizes the clinical condition of "hyperinsulinism"

consequent to the hyperactivity of the islands of Langerhans (13). These facts make it apparent that *insulin exerts a ketolytic effect when it is antiglycogenolytic in action and, conversely, it exerts a ketogenic effect when it is glycogenolytic with regard to the liver.*

If these deductions are correct then any substance which will inhibit hepatic glycogenolysis should also prevent the production of ketones. This is borne out by our observations on the ergotaminized animal (fig. 1, *C*). It is now well established that ergotamine will prevent the hepatic glycogenolysis produced by various agents, especially epinephrin. The observations of Corkill, Marks and Soskin indicate that this antiglycogenolytic effect is more specific for the liver than it is for muscle since they found that the glycogenolytic effect of epinephrin on muscle is not annulled by ergotamine (14). Thus we find that a drug which exerts a specific antiglycogenolytic action on the liver also inhibits the production of the acetone bodies which are normally produced by the administration of an extract of the anterior hypophysis.

These observations are in accord with a working hypothesis which we presented in a previous report. According to this hypothesis, the production of acetone bodies is the result of an increased catabolism of non-carbohydrate foodstuffs (gluconeogenesis) in the presence of a *relative decrease in the carbohydrate available for oxidation by the cells of the liver.* The latter may result from carbohydrate starvation (as in "hunger diabetes") or from a persistently accelerated hepatic glycogenolysis (as in "pancreatic diabetes"). Thus, both "*non-utilization*" and "*over-production*" of carbohydrate are believed to occur in the *liver*, the carbohydrate utilization of the extrahepatic tissues remaining essentially undisturbed. From this point of view it is obvious that the muscles of the diabetic organism will be able to utilize acetone bodies at the same rate as those of the normal animal (6) since these substances can be completely oxidised in the presence of adequate carbohydrate oxidation. It is also obvious that if the liver glycogen is replenished by the administration of glucose, ketone production should cease. This is a common clinical and experimental experience in nearly all conditions associated with ketosis. The chief exception to this is the observation that the ketonuria of the depancreatized dog is not decreased by the administration of a single dose of glucose (15). However, we have observed that even in the depancreatized dog, ketosis can be inhibited by the prolonged continuous administration of glucose (16).

SUMMARY AND CONCLUSIONS

1. Insulin, a physiological agent, and ergotamine, a pharmacological agent, inhibit the production of acetone bodies which normally result from the administration of a neutralized alkaline extract of the anterior hypophysis.

2. Since the hypophyseal extract is effective only in the presence of the liver, it is probable that these substances exert their antiketogenic effects on the liver.

3. The mechanism of the antiketogenic action of insulin and ergotamine is discussed, and it is suggested that these substances owe this action to their antiglycogenolytic function.

4. The mechanism of ketone formation in the liver is discussed.

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REFERENCES

(1) CHAIKOFF, I. L., J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON. This Journal **74:** 36, 1925.
(2) KILLIAN, J. A. J. Lab. Clin. Med. **11:** 1132, 1926.
(3) SOSKIN, S. J. Nutrition **3:** 89, 1930.
(4) SOSKIN, S., I. A. MIRSKY, L. M. ZIMMERMAN AND N. CROHN. This Journal **114:** 110, 1935.
(5) MIRSKY, I. A. This Journal **115:** 424, 1936.
(6) CHAIKOFF, I. L. AND S. SOSKIN. This Journal **87:** 58, 1928–29.
(7) MAJOR, S. G. AND F. C. MANN. This Journal **102:** 409, 1932.
(8) MACLEOD, J. J. R., E. C. NOBLE AND M. K. O'BRIEN. Trans. Roy. Soc. Can. Sect. V **18:** 129, 1924.
(9) SAHYUN, M. AND J. M. LUCK. J. Biol. Chem. **85:** 1, 1929–30.
(10) BURN, J. H. AND H. W. LING. J. Physiol. **65:** 191, 1928.
(11) COLLIP, J. B. J. Biol. Chem. **55:** xxviii, 1923.
(12) LOW, A. AND A. KRCMA. Wien. Arch. Inn. Med. **24:** 455, 1934.
(13) HARRIS, S. Am. J. Dig. Dis. and Nutrition **2:** 557, 1935.
(14) CORKILL, A. B., H. P. MARKS AND S. SOSKIN. J. Physiol. **83:** 26, 1934.
(15) CHAIKOFF, I. L. AND J. J. WEBER. Proc. Soc. Exper. Biol. Med. **25:** 212, 1927.
(16) MIRSKY, I. A., J. D. HEIMAN AND C. STRIKER. (In preparation.)

THE INFLUENCE OF PARTIAL PRESSURE OF O_2 ON BODY TEMPERATURE

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In 1927 Behague and collaborators observed that under the influence of a diminished barometric pressure the temperature in rabbits suffered a decrease. This phenomenon has since been confirmed by Chevillard and Mayer (1935) for various animals. In view of the fact that in the recent literature (Margaria, 1928; Winterstein, 1934, and others) it was claimed that not all effects of lowering of barometric pressure are due to the decrease in the partial pressure of O_2, a statement which is in strict contradiction to the older observations of Bert (1878), Douglas, Haldane, Henderson and Schneider (1913), and others, it seemed desirable to re-investigate this problem. The fall in temperature occurring in the low pressure chamber, as well as in air which is diluted with nitrogen, seemed to lend itself to such a quantitative investigation.

METHOD. A first series of experiments was carried out in a low pressure chamber at a barometric pressure of 460 mm. Hg. The temperature drop did not amount to more than about 1°C., and seemed insufficient for a quantitative study in view of the slight spontaneous variations in temperature. A further lowering of the barometric pressure was poorly tolerated by the animals, but the addition of 9 per cent CO_2 to the air allowed us to lower the barometric pressure to 225 mm. Hg without producing acute symptoms. The same CO_2-tension was used in every series of experiments. The animals were kept in the low pressure chamber for 45 minutes. The temperature before and afterwards was measured rectally.

RESULTS. Figure 1a shows the course of the mean temperature of ten animals subjected to a barometric pressure of 225 mm. Hg for 45 minutes. The vertical lines drawn through each point of the graph indicate the maximal variations. It is obvious that a considerable drop in temperature occurs under these circumstances. The normal temperature is reached in approximately 60 to 100 minutes.

In a second group of experiments the low pressure chamber was again evacuated to 225 mm. Hg, but then nitrogen was added until atmospheric

pressure was attained. Thus was obtained a condition of normal atmospheric pressure with a reduced O_2-tension of the same value as in the first group of experiments. The result of this experiment carried out with eight guinea pigs (fig. 1b) shows that almost exactly the same drop in temperature occurs under these circumstances and that approximately the same time is required for a complete recovery. The mean temperature fall obtained immediately after the exposure of the animals to a reduced partial pressure of O_2 was 2.6°C. in the first group, and 2.4°C. in the second. The standard deviation in the first group was 0.23°C., and in the second group 0.16°C., the standard errors being 0.07° and 0.06° respectively. This quantitative agreement seems to prove conclusively that the lowering of barometric pressure causes a drop in temperature by the reduction of the partial pressure of O_2. In order to further elucidate

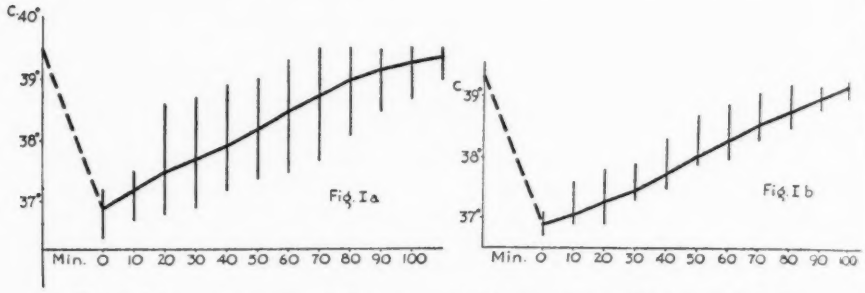

Fig. 1. The curves represent the mean rectal temperatures of guinea pigs before and after exposure to the same decrease in partial pressure of O_2 with (fig. 1a) and without (fig. 1b) decrease in barometric pressure. The vertical lines drawn through each point represent the maximal variations.

the significance of the partial pressure of O_2 under these conditions the following experiment was carried out. Guinea pigs were placed in the low pressure chamber, the pressure was reduced to 225 mm. Hg as before, and then O_2 was added until atmospheric pressure was reëstablished. The tank was then evacuated again to a pressure of 225 mm. Hg, thereby reducing the partial pressure of O_2 to 149 mm. Hg. Thus the condition was obtained that the barometric pressure was the same as in the first group of experiments, but the partial pressure of O_2 was slightly higher than in air at normal atmospheric pressure. The experiments carried out with eight guinea pigs indicated that the body temperature remained unaltered. The maximal differences in temperature were 0.2°C. This proves that it is the reduction of the partial pressure of O_2 that lowers the body temperature, and that the alteration of the barometric pressure per se has no effect whatever.

SUMMARY

Three groups of experiments are reported in which guinea pigs were subjected: *a*, to a lowered barometric pressure, normal air being used so that atmospheric pressure and partial pressure of O_2 were diminished; *b*, to a reduction in the partial pressure of O_2 at normal barometric pressure; *c*, to a reduction of barometric pressure at normal partial pressure of O_2.

The temperature remained normal in group *c* and was lowered in *b* and *c* to the same degree, thereby showing that the partial pressure of O_2 influences the body temperature and that alterations in the barometric pressure are without any effect.

REFERENCES

BEHAGUE, P., GARSAUX AND C. RICHET. Compt. rend. Soc. biol. **96:** 766, 1927.
BERT, P. La barometrique pressure, 1878.
CHEVILLARD, L. AND A. MAYER. Annal. de physiol. **11:** 225, 1935.
DOUGLAS, HALDANE, HENDERSON AND SCHNEIDER. Phil. Trans. B. **203:** 185, 1912.
MARGARIA, R. Arch. di. sci. biol. **11:** 425, 453, 1928.
WINTERSTEIN, H. Acta aerophysiol. **1:** No. 2, 3, 1934.

THE VASOMOTOR EFFECT OF ACETYLCHOLINE ON THE STOMACH OF THE DOG[1]

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In a recent paper, we reported that the rat's stomach perfused with solutions of acetylcholine (a.c.) responded with vasoconstriction (1). Perfusion of isolated segments of mesenteric and gastric arteries and veins from dog and man resulted in constriction when a.c. was added to the perfusion fluid (2). In these two papers the literature pertaining to this subject was reviewed and discussed. Our findings of vasoconstriction in the vascular bed of the rat's stomach due to a.c. (1) prompted us to examine this effect under more physiological conditions, using the stomach of the dog, which is more similar to that of the human, anatomically and physiologically. Our interest in this problem was promoted by the work of Dale and Feldberg, announcing the presence of increased amounts of a.c. in the splenic blood after stimulation of the vagi (3). If the stomach of the dog showed a considerable degree of vasoconstriction to small doses of a.c., a possible explanation for the genesis of gastritis and peptic ulcer might be entertained. This belief was strengthened by the fact that the stomachs of ulcer patients frequently display permanent and increased activity, which may be due to a more continuous production in these stomachs of a.c. than occurs in normal stomachs (1).

METHODS. Dogs were placed under ether or pentobarbital anesthesia. The following experimental procedures were employed: 1, perfusion of the excised stomach of a smaller dog by a larger perfuser dog and measurement of the rate of flow through the small stomach; 2, measurement of the rate of bloodflow through an innervated stomach in situ; and 3, oncometry of viviperfused stomachs.

Preparation: ad 1. The method previously described by Lim, Necheles and Ni (4) was employed, except that Payr cannulas were used for intima to intima anastomosis of the blood vessels. The celiac artery and the portal vein of the excised preparation were connected to the common

[1] A report of this work was presented at the 48th Annual Meeting of the American Physiological Society, 1936.

Acetylcholine was supplied by Hoffmann-LaRoche

carotid artery and external jugular vein respectively of the perfuser dog. The hepatic artery of the excised stomach was used for injection of a.c. The superior mesenteric vein of the isolated stomach was prepared and utilized for measuring the venous outflow from the perfused stomach, using a pipette and stopwatch (method of Barcroft and Kato, 5). In determining the rate of flow, the portal vein was closed with a bulldog clamp and the superior mesenteric vein opened simultaneously, and the time taken for a given volume of venous blood from the stomach to flow into the pipette. After the reading was taken, the clamp was removed from the portal vein and the blood from the pipette permitted to return into the portal-external jugular vein. A few drops of sodium citrate in the pipette prevented clotting. The injection of a.c. into the hepatic artery was made either by constant injection (modified Woodyatt pump), or with a syringe and fine needle (1 minute injection time). A balloon in the perfused stomach, connected to a water manometer, recorded gastric motility. A glass tube tied into the pyloric canal permitted the evacuation of gastric secretion. The blood pressure of the perfuser dog was recorded by means of a mercury manometer.

Ad 2. The entire intestinal tract from the duodenum down was removed without interfering with the blood or nervous supply to the stomach. The superior mesenteric vein was isolated and utilized as in 1 for measuring the rate of bloodflow through the stomach. The splenic artery was utilized for injection of a.c.

Ad 3. A simple metal oncometer was used. The preparation of the stomach was as in 1, but no balloon was inserted into the stomach, because motility would have vitiated the recording of volume changes of the stomach; a sensitive Palmer piston recorder was used for the latter purpose. A tube in the pylorus was opened from time to time to enable the stomach to expel its secretion. The excised stomachs in 1 and 3 were anoxemic for 5 to 12 minutes.

The experiments lasted from 2 to 6 hours. Only such tests are being reported in which constant control readings of bloodflow were obtained, and in which the bloodflow had returned to the control level after the injection of a.c. was stopped. No readings are reported which were taken during gastric contractions. All experiments in which the stomach did not respond to adrenaline or larger doses of a.c. with vasoconstriction and motility respectively were discarded. The differences in bloodflow in table 1 are expressed as percentage change from preceding controls which had to be constant for a period of 10 minutes; the doses of a.c. are expressed in gammas. The limits of error of the method for measuring bloodflow including physical and biological variations, we consider to be within ± 10 per cent, and only values beyond this are significant. The secretion of the stomach consisted of mucus, but was never so copious as

TABLE 1

Effect of acetylcholine on bloodflow through the dog's stomach

ACETYL CHOLINE GAMMA PER MINUTE	± PER CENT CHANGE OF VENOUS OUTFLOW
Denervated stomach	
Expt. 1	
0.005	0
0.01	−36
0.25	−27
1.0	−33
5.0	+24
20.0	+37
40.0	+51
Expt. 2	
0.002	−10
0.004	+28
20.0	+65
Expt. 3	
0.001	−31
0.01	+25
2.0	+21
Expt. 4	
0.0005	−17
0.001	+13
0.002	+41
Expt. 5	
0.0001	−64
0.004	−3
0.008	−5
0.2	−13
20.0	+33
1000.0	+67
Expt. 6	
1.25	−25
5.0	+25
10.0	+29
Expt. 7	
0.1	0
1.0	−14
2.0	−6
4.0	−3
10.0	+31
100.0	+21

ACETYL CHOLINE GAMMA PER MINUTE	± PER CENT CHANGE OF VENOUS OUTFLOW
Expt. 8	
0.1	−96
0.2	−59
0.3	−58
0.4	−37
1.0	−56
200.0	+26
Expt. 9	
0.1	−20
5.0	−65
10.0	+3
25.0	+68
50.0	+66
Expt. 10	
0.01	0
0.1	−13
0.2	−19
5.0	−16
7.5	+35
Expt. 11	
0.2	0
1.0	−5
2.0	−10
10.0	+13
20.0	+17
50.0	+23
200.0	+29
Expt. 12	
0.02	+4
0.4	+3
0.5	−12
0.8	−12
1.0	+19
2.0	+14
20.0	+5

ACETYL CHOLINE GAMMA PER MINUTE	± PER CENT CHANGE OF VENOUS OUTFLOW
Expt. 13	
0.1	0
1.0	−13
10.0	−20
50.0	+18
Innervated stomach	
Expt. 14	
0.1	−17
0.2	0
0.5	+11
Expt. 15	
0.003	−53
0.3	+22
Expt. 16	
0.01	−13
0.05	+21
0.1	+13
Expt. 17	
0.01	0
0.03	−10
0.06	−10
0.1	−8
0.24	0
0.3	+25
Denervated stomach	
Expt. N1	
0.2	−4
2.0	0
4.0	−10
20.0	−8
Expt. N2	
0.00002	−7
0.00005	−11
0.002	−25

ACETYL CHOLINE GAMMA PER MINUTE	± PER CENT CHANGE OF VENOUS OUTFLOW
Expt. N3	
0.001	−7
0.1	−21
0.2	−34
5.0	−19
10.0	−19
Expt. P1	
40.0	+70
Expt. P2	
0.01	−5
1.0	−3
10.0	+11
100.0	+33
200.0	+47
Expt. P3	
0.0001	−6
10.0	+26
Expt. P4	
8.0	+40
Expt. P5	
2.0	+16
Innervated stomach	
Expt. P6	
0.1	+16
1.2	+48
Expt. P7	
0.000003	+5
0.00005	0
0.03	+42
0.3	+48
26.0	+53

to warrant a correction for the rate of perfusion as measured by the venous outflow.

RESULTS. The experiments on the denervated and innervated stomach are reported in table 1. All of the stomachs responded to relatively large doses of a.c. with muscular contraction. The sensitivity and reactivity of the individual preparations varied, and therefore no averages are presented. Nevertheless most of the experiments agree in essential features. Smallest concentrations of a.c. did not produce any significant effect on the rate of gastric perfusion; e.g., (expt. 1) 0.005 gamma, (expt. 17) 0.01 gamma, etc. With increasing doses of a.c. a diminution of the rate of gastric perfusion occurred; e.g., expt. 1 with 0.01 gamma, minus 36 per cent, etc.; further increase of the dose of a.c. produced a reversal of the preceding effect, and a greater rate of perfusion was recorded; e.g., expt. 1 with 5.0 gamma, plus 24 per cent, etc. A response to a.c. as described above was observed with denervated stomachs in experiments 1 to 13 and with innervated stomachs in experiments 14 to 17. In a number of experiments the change from diminished to increased perfusion rate could be demonstrated to pass through a zero point; as in experiments 5 with 0.004 gamma, 7 with 2.0 gamma, 9 with 10.0 gamma, 14 with 0.2 gamma and 17 with 0.24 gamma. It was noted sometimes that larger doses of a.c. produced less increase of flow than smaller doses; e.g., in experiment 7,100 gamma of a.c. produced +21 per cent increase of flow, while 10 gamma produced +31 per cent. This is in line with our observations on the rat's stomach (1) and on isolated bloodvessels (2) where we found that relatively high concentrations of a.c. diminished the sensitivity of the stomach and often killed the preparation.

In experiments N1 to N3 only diminished rate of perfusion was obtained and in experiments P1 to P7 only increased rate of perfusion was seen following a.c. It is assumed that in these latter two groups either too small or too large doses were given, so that the entire range of a possible biphasic (or reversed) reaction was not tested. This was not due to experimental error, but rather to the fact that in the process of evaluation many results had to be discarded, either because controls were found to be unsatisfactory or careful checking of simultaneous points on the records revealed that gastric motility occurred at the time the readings of blood-flow were taken. In some cases the preparation died before all concentrations of a.c. could be tested. Therefore we believe that experiments 1 to 17, representing 63 per cent of all experiments done, are typical of a biphasic response to a.c. in the denervated and innervated stomach.

Oncometry. Four experiments were performed and since the results were uniform, they are presented in one table (table 2).

Doses from 0.01 to 100 gamma increased the volume of the stomach. Due to technical difficulties the rate of perfusion could not be measured

simultaneously with the gastric volume. In 3 cases the increase of volume was preceded by a slight drop. This drop was transient (30 seconds) and not comparable to the biphasic effect reported above, because in the experiments in which the rate of flow was determined, the increased or decreased rate of flow was maintained as long as the injection of a.c. lasted.

Discussion and conclusions. Hunt (6) and Fleisch (7) have described a biphasic effect of a.c. on bloodvessels of dogs and cats. Hunt perfused the liver, Fleisch used mesenteric veins. Both of them obtained constriction with large doses and dilatation with smaller doses of a.c. Fleisch stresses the importance of handling the veins with extreme care and of using preparations with intact innervation (in situ), because otherwise only contraction is obtained with a.c. While our results agree with those of Hunt and Fleisch in that we obtained a biphasic response to a.c., they are opposed to theirs in two important points. With methods 1 and

TABLE 2

Oncometry of the denervated perfused stomach

ACETYLCHOLINE IN GAMMA	STOMACH VOLUME
0.01	No change
0.4	Increased +
1.0	Increased (+)
2.2	Increased +
3.0	Increased +. Preceded by slight fall
4.0	Increased ++
10.0	Increased +. Preceded by slight fall
12.0	Increased +++
15.0	Increased ++
100.0	Increased ++. Preceded by slight fall

2, we observed contraction with small doses and dilatation with increasing doses, and denervation of the stomach did not affect our results. This last finding convinced us that innervation was not necessary for biphasic response and therefore in perfusion experiments on mesenteric and gastric veins and arteries of dogs we did not attempt to use innervated vessels. Although similar care was taken as Fleisch did in the preparation of the vessels, only contraction was obtained with a.c., which was more marked in the veins than in the arteries; and no biphasic response was seen (2).

The question arises as to where in the vascular bed of the stomach contraction and dilatation, respectively, occurred in our experiments. In the oncometric experiments doses from 0.01 to 100 gamma resulted in increased gastric volume. Since the rate of perfusion could not be determined in the oncometric experiments we can only speculate about their interpretation. Assuming that in some of them slowing and in others an

increase of the rate of perfusion occurred, the greater stomach volume in both cases can be interpreted to mean that capillary dilatation occurred in both but that a considerable degree of constriction of veins occurred only with smaller doses. That the biphasic effect of a.c. is not a phenomenon of physiological rebound was demonstrated by constant injection, by which the effects of diminished or increased rate of gastric perfusion could be maintained as long as the injection lasted (5–20 min.). Contractions of the stomach did not produce constant effects on bloodflow through the stomach. This is in agreement with previous observations of one of us (4), but opposed to the findings of Burton Opitz, who reported increased rate of flow following gastric contractions (8). There is a remote possibility, however, that contractions of the gastric muscularis mucosa, not recorded by the balloon-water manometer method, may change the rate of gastric blood flow. In a number of experiments in which the bloodflow was measured, an increased rate of perfusion was followed by a slightly decreased rate of perfusion and vice versa.

In relation to the problem of the genesis of peptic ulcer the question arises as to what concentration of a.c. may occur physiologically in the stomach: that producing a decrease or that producing an increase of the bloodflow through this organ. Dale and Feldberg (3) determined quantitatively that the amount of a.c. liberated in the dog's stomach following stimulation of the vagi is approximately 2 gamma per cent of a.c. They used eserine in order to inhibit destruction of the a.c. by blood and tissue esterase. We cannot calculate, with exactness, the degree of dilution of the a.c. injected in our experiments, and we do not know the rate of its destruction. It seems plausible, however, to assume that Dale and Feldberg's value of 2 gamma per cent is within that range which in most of our experiments produced diminution of the rate of gastric perfusion. Since this decrease of bloodflow amounts to considerable values in a number of experiments (minus 36, 31, 64, 25, 96, 65, 19, 20 and 53 per cent in experiments 1, 3, 5, 6, 8, 9, 10, 13 and 15 respectively) and since it can be maintained as long as the injection of a.c. is continued, we conclude that this effect of a.c. may be an important factor in the genesis of ulcer, in such areas in which end arteries are found, as in the distal part of the lesser curvature and in the duodenal cap (9–13). The biological variation of the effective doses of a.c., the biphasic effect in 65 per cent of our experiments, and prevalence of vasoconstriction or vasodilatation in the rest of our experiments, tends to explain individual predisposition to peptic ulcer.

SUMMARY

The effect of acetylcholine on the rate of bloodflow through denervated and innervated stomachs of 27 dogs was determined. In 17 of these

experiments (63 per cent) small doses of acetylcholine decreased the rate of bloodflow, while larger doses increased it. In 3 experiments, only diminution of flow, and in 7 experiments, only increase of flow was observed. The bearing of these results on the genesis of peptic ulcer is discussed.

REFERENCES

(1) Necheles, H., R. Frank, W. Kaye and E. Rosenman. This Journal **114:** 685, 1936.
(2) Kohn, R., P. Levitsky, A. A. Strauss, S. Strauss and H. Necheles. In press, Arch. Internat. de Pharmacodyn. 1936.
(3) Dale, H. H. and W. Feldberg. J. Physiol. **81:** 320, 1934.
(4) Lim, R. K. S., H. Necheles and T. G. Ni. Chinese J. Physiol. **1:** 381, 1927.
(5) Barcroft, J. and T. Kato. Phil. Trans. Roy. Soc., B. **207:** 149, 1916.
(6) Hunt, R. This Journal **45:** 197, 231, 1918.
(7) Fleisch, A. Pflüger's Arch. **228:** 351, 1931.
(8) Burton-Opitz, R. Pflüger's Arch. **135:** 204, 1910.
(9) Disse, J. Arch. f. mikr. Anat. **63:** 512, 1904.
(10) Reeves, T. B. Surg., Gynec. and Obst. **30:** 374, 1920.
(11) Jatrou, S. Deutsch. Ztschr. f. Chir. **159:** 196, 1920.
(12) Hoffmann, L. and K. Nather. Arch. f. klin. Chir. **115:** 650, 1921.
(13) Berlet, K. Frankf. Ztschr. f. Path. **30:** 472, 1924.

THE CHLORIDE AND ALKALI CONTENT OF THE DUODENAL SECRETIONS AND THEIR RELATION TO GASTRIC ACIDITY AND EMPTYING TIME[1]

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Ever since Boldyreff (1914) showed that the acidity of a volume of acid placed in a dog's stomach was rapidly reduced, much controversy has raged around the question of the factors regulating acidity. Most of the published work relating to this has been done on animals, and comparatively little on man.

Working entirely on human subjects, one of us has shown *a*, that the individual variations in acidity found in an ordinary test-meal depend more on the ability to reduce acidity than on the secretion of acid (Apperly, 1923); *b*, that the reduction of acidity depends far more on dilution than on neutralization (Apperly, 1926), a fact since confirmed in dogs by the ingenious experiments of Wilhelmj, Henrich, Neigus and Hill (1933-35); *c*, that, in spite of the fact that the chloride concentration of pure gastric juice is about 0.170 N (Hollander and Cowgill, 1931), the total chloride concentration *finally* reached in a test-meal—though differing widely in different individuals (Apperly and Semmens, 1928)—is constant for each individual and is unaffected by the addition to the meal of hydrochloric acid, sodium chloride, or sodium bicarbonate in different amounts, or even by gastroenterostomy (Apperly, 1926); *d*, that the emptying time of the stomach varies with the final total chloride (Apperly, 1926) and with the acidity (Apperly and Semmens, 1928), e.g., those people with a low acidity and final total chloride generally empty rapidly.

From the above facts the following deductions appear justifiable:

1. The fall of total chloride and acidity in gastric contents is caused by dilution with a solution of lower chloride concentration than that found in pure gastric juice.

2. The greatest reductions in total chloride of the gastric contents are caused by either *a*, a greater volume of diluting fluids (as suggested by Wilhelmj et al., 1933, 1935), or *b*, dilution with those solutions which contain the lowest concentrations of chloride.

[1] Part of the expenses connected with this work was donated by Parke, Davis Co. of Detroit.

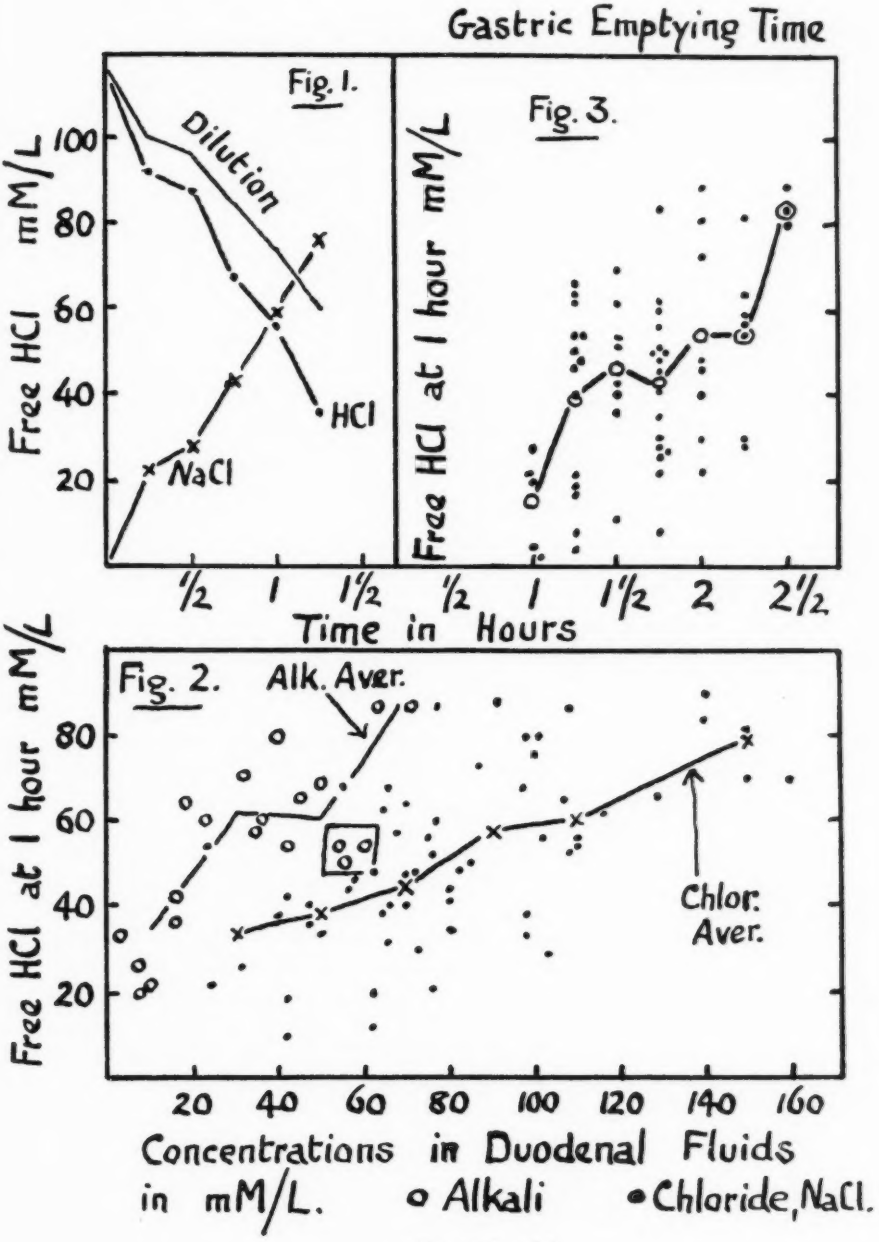
It was hoped that a demonstration of the above would throw some light on the difficult problem of the regulation of emptying time.

The investigation of these relationships in animals can be made directly by operative methods designed for collecting separately the secretions from fundus, pyloric end, duodenum, pancreas, intestine and gall bladder. But in man indirect methods must be used. In these experiments we have again used the hydrochloric acid "test-meal," in spite of the fact that in some cases an unknown amount of hydrochloric acid is secreted by the stomach even in the presence of the acid introduced, as has been shown by Apperly and Norris (1930), Wilhelmj et al. (1933), (and denied by Pavlov, 1910; Ivy and Whitlow, 1922; MacLean et al., 1928). This secreted acid may be masked by more or less neutralization by alkali of pyloric or duodenal origin, producing a certain amount of neutral chloride. It is, however, impossible in these human cases to differentiate between that neutral chloride which is secreted as such, and that which results from neutralization of secreted acid. These considerations, however, do not appear to affect our final conclusions.

Methods. Experiments were made on a number of healthy students and hospital patients in the manner first used by Apperly in 1923. Into the stomach of each were placed 250 cc. of warmed 0.110 N hydrochloric acid, to which was added some indifferent substance which could be easily estimated, such as glucose, phenol-sulphonephthalein, urea, or ferric chloride, in order to indicate the dilution of the stomach contents.

Each experiment was carried out on the fasting subject. The Rehfuss tube was first swallowed, the fasting contents withdrawn by a syringe attached to the tube, the stomach washed out with water till clear, and the acid mixture then run in from a large pipette attached to the Rehfuss tube. At this moment the subject was instructed to expectorate all saliva into a beaker until the end of the experiment. This saliva contained no titratable alkali when phenolphthalein was used as indicator. At fifteen-minute intervals 10 cc. samples were withdrawn from the stomach, after mixing the contents thoroughly by moving the piston of the syringe back and forth seven or eight times. In each sample there were estimated the concentrations of total chloride, acid and glucose (or urea, dye, or iron). The acid was estimated by titration with fresh 0.01 N sodium hydroxide, using phenolphthalein as indicator. Total chloride was estimated by Volhard's method and neutral chloride by difference. When glucose was used, MacLean's method was employed: for urea, MacLean's urease method; for ferric chloride, the method of San Yin Wong (1928) for phenolsulphonephthalein a Klett colorimeter. In none of the subjects examined did the fasting contents contain glucose, urea, or iron.

Results. The results varied, but the majority were similar to that shown in figure 1. This shows that the concentration of acid falls more


Gastric Emptying Time
Fig. 1.
Dilution
HCl
NaCl
Free HCl mM/L
100
80
60
40
20
½
1
1½
Time in Hours
Fig. 3.
Free HCl at 1 hour mM/L
½
1
1½
2
2½
Fig. 2.
Alk. Aver.
Chlor. Aver.
Free HCl at 1 hour mM/L
80
60
40
20
20
40
60
80
100
120
140
160
Concentrations in Duodenal Fluids
in mM/L.
o Alkali
• Chloride, NaCl.

Figs. 1, 2 and 3

rapidly than does that of the glucose (or urea, dye, etc.). This indicates that at, say, one hour after the administration of the acid mixture, the acidity has been reduced from its original strength chiefly by dilution but partly by neutralization of acid. Further, as the acidity falls, there is a rise of the neutral chlorides, of which obviously only a small part can be accounted for by neutralization of acid. The remainder must have been secreted as such by the stomach or regurgitated from the duodenum.

In order to estimate the original chloride and alkali concentrations in the diluting fluids the following formula has been used:

$$\frac{k}{K+A} = \frac{G-g}{G} = \frac{H-h}{H+A}$$

where k = the sodium chloride, h = the acid, and g = the glucose (or urea, etc.) concentrations in the contents withdrawn from the stomach at the moment selected: H = the acid, and G = the glucose concentrations in the mixture originally placed in the stomach, K = the sodium chloride, and A = the alkali concentrations in the mixed diluting fluids, whether of gastric or duodenal origin.

In some of the earlier experiments no glucose or dye was used, and the values of K are approximations based on the assumption that $K = k\frac{H}{H-h}$. Since in no case did the acidity and dilution curves diverge widely, and in a number of instances even coincided, this approximation is near enough for our purposes. It is probable that the error does not exceed 0.004 to 0.006 N.

In addition to these "typical" cases (illustrated in fig. 1), there were some in which, as already stated, the acidity and dilution curves coincided, indicating an absence of alkali in the diluting fluids. In a few others, the dilution curve fell more rapidly than the acidity curve, indicating that, as already pointed out, gastric acid can be, and occasionally is, secreted into the stomach in the presence of a foreign acid therein. These cases have been discarded for the purposes of this enquiry.

In figure 2 the results from experiments on 59 individuals are plotted. From each experiment the acidity at one hour is plotted against the corresponding calculated values of K, the original concentration of neutral chloride in the diluting fluids secreted by the stomach or duodenum, or both. The acidity in 21 experiments is also plotted against the corresponding calculated alkali concentration of the diluting fluids.

These experiments show that, in general, the concentration of acid in the stomach one hour after acid has been placed therein varies with a, the concentration of both neutral chloride and alkali in the diluting fluids (fig. 2), and b, the emptying time of the stomach (fig. 3). The latter had been demonstrated previously in another way (Apperly and Semmens, 1928).

DISCUSSION. The acidity of the contents of the stomach at any moment, say one hour, after a standard volume and concentration of acid has been placed therein is of course the resultant of the mixture of the acid then remaining in the stomach with diluting fluids secreted by the stomach or regurgitated from the duodenum.

The volume of acid remaining in the stomach at the moment selected varies with the emptying time. If the latter is rapid, so that only a small volume of acid remains, while the volume of diluting fluids remains practically the same, then acidity will be low. That acidity varies closely with emptying time (fig. 3) in spite of other factors, suggests that emptying time is the most important factor in regulating acidity.

As regards the diluting fluids, containing neutral chloride and alkali, there is still some controversy as to whether they are wholly secreted by the stomach mucosa (as maintained by MacLean and others, 1928) or are mostly regurgitated from the duodenum (Bolton and Goodhart, 1931; Apperly and Norris, 1930, and others). In dogs, however, Wilhelmj and his colleagues (1933, 1935) have shown that both these organs contribute diluting fluids, but that the part played by the stomach is relatively small. In man the little (and conflicting) evidence regarding the neutral chloride seems to indicate a diminishing concentration in the following order, viz., stomach, pancreas, bile and succus entericus. In dogs Wilhelmj et al. found the neutral chloride content of pyloric pouches to be 0.085 to 0.135 N. while fluids from the duodenum, pancreas, and intestine were 0.065 to 0.100 N. This diminishing chloride concentration in the lower duodenal levels will be referred to later.

What now regulates emptying time? The well known work of Cannon showed that acid in the duodenum closes the pylorus, and many authors have demonstrated that the elimination of pancreatic alkali causes the stomach to empty more slowly (Boldyreff, 1914; Apperly and Cameron, 1923; Wilhelmj et al., 1933). It would appear that the emptying rate of the stomach depends upon the duodenal mechanism for neutralizing or diluting acid entering the duodenum from the stomach, and that rapid emptying would be brought about by either a higher concentration of alkali in, or a larger volume of, the duodenal fluids. The former of these possibilities is eliminated by the fact that, as shown in figure 2, in general the higher concentrations of alkali in the diluting fluids are associated with a higher acidity, which, as figure 3 shows, means slower emptying, although it is true that, in a few cases (those enclosed in a square in figure 2) the regurgitation into the stomach of fluids with increased alkalinity had the reverse effect, which is not surprising. We are therefore driven to assume that acid entering the duodenum is diluted by larger volumes of regurgitated fluids. Such larger volumes indicate a regurgitation from the lower levels of the duodenum or upper jejunum, levels at which chlo-

rides are secreted, according to evidence discussed above, at lower concentrations. If this reasoning is correct we would therefore expect the lower concentrations of neutral chloride in the diluting fluids to be associated with rapid gastric emptying and therefore low acidity. Our experiments show that this is so.

CONCLUSIONS

1. When hydrochloric acid is introduced into the human stomach it is found that:

a. The acidity is reduced, chiefly by dilution and to only a small extent by neutralization.

b. The rate of reduction of acidity varies directly with the rate at which the stomach empties.

c. The rate of reduction of acidity varies inversely as the concentration of neutral chloride and alkali in the fluids which dilute the gastric contents.

d. The concentration of alkali in the human duodenum varies from 0 to 0.075N.

2. A consideration of these results and the work of others leads us to believe that the diluting fluids of lower salt and alkali concentrations are derived from the lower levels of the duodeno-jejunum and are regurgitated in greater volumes, producing a more rapid reduction of acid in the duodenum and more frequent opening of the pylorus. The stomach contents are, therefore, more rapidly emptied and diluted. The result is a low acidity of the gastric contents. High acidity is the result of an opposite set of conditions.

REFERENCES

APPERLY, F. L. Med. J. Australia **1:** 33, 1923.
British J. Exper. Path. **7:** 111, 1926.

APPERLY, F. L. AND G. R. CAMERON. Med. J. Australia **1:** 521, 1923.

APPERLY, F. L. AND J. H. NORRIS. J. Physiol. **70:** 158, 1930.

APPERLY, F. L. AND K. M. SEMMENS. Med. J. Australia **2:** 226, 1928; **2:** 237, 1928.

BOLDYREFF, W. Quart. J. Exper. Physiol. **8:** 1, 1914.

BOLTON, C. AND G. W. GOODHART. J. Physiol. **73:** 115, 1931.

HOLLANDER, F. AND S. R. COWGILL. J. Biol. Chem. **91:** 151, 1931.

IVY, A. C. AND J. E. WHITLOW. This Journal **60:** 578, 1922.

MACLEAN, H. AND W. J. GRIFFITHS. J. Physiol. **65:** 63, 1928; **66:** 356, 1928.

MACLEAN, H., W. J. GRIFFITHS AND B. W. WILLIAMS. J. Physiol. **65:** 67, 1928.

PAVLOV, I. P. The work of the digestive glands (Thompson's translation). Griffin, London, 1910.

SAN YIN WONG. J. Biol. Chem. **77:** 409, 1928.

WILHELMJ, C. M., L. C. HENRICH, I. NEIGUS AND F. C. HILL. This Journal **106:** 381, 1933; **112:** 15, 1935.

THE EFFECT OF GOOD ELECTRICAL CONDUCTORS INTRODUCED NEAR THE HEART ON THE ELECTROCARDIOGRAM

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Recently, we have shown that the tissues in contact with the heart differ sufficiently in their electrical conductivity to give some of them a greater rôle than others in carrying away the currents which the heart generates (Katz and Korey). It was shown further that certain regions of the heart gain a decided advantage over the rest when they are connected through a good electrical conductor to a region on the chest nearer to one of the two recording electrodes employed (Katz, Gutman and Ocko).

These observations indicated the importance of the electrical properties of tissues adjacent to the heart in determining the electrical field set up by it. In the present communication observations are reported on the effect of introducing good metallic or non-metallic electrical conductors adjacent to the heart on the electrical records obtained from it. The action of these good conductors is not simple, but as was soon apparent, they act in at least three different manners, viz: 1, by offering shunts for the currents generated by the heart; 2, by altering the relative contribution of the various regions of the heart to the recorded electrical curves, and 3, by creating electrical stresses, the result of frictional electricity between dissimilar conductors. The relative importance of these actions was found to depend on the method of recording the electrical curves, on the presence or absence of regions of injury in the heart, and on the nature of the electrical conductor introduced.

METHODS. Dogs anesthetized with sodium barbital were used. The chest was opened, and artificial respiration instituted. The heart was fixed in place by stay sutures fastened to the pericardium. In most experiments the pericardium was opened. The distant recording electrodes were inserted subcutaneously in the two forelimbs and the left hindlimb when standard electrocardiograms were obtained. These electrodes consisted of German silver plates covered with gauze soaked in saline. The electrodes used in recording directly from two points on the heart whether from two injured regions, from two uninjured regions, or from one of each

were similar to those previously described, as was also the manner of producing injury (Jochim, Katz and Mayne). The technique used in obtaining records with one electrode on the heart and another at a distance from it was also similar to that described in the aforementioned communication.

The following good conductors were introduced in various experiments:

a. Gauze pads soaked in isotonic saline and about 1 cm. in diameter and several millimeters in thickness were placed on the ventricles when a pair of distant recording electrodes was used; and around a recording electrode on the heart when a pair of direct recording electrodes or a combination of direct and distant recording electrodes was used.

b. Warm isotonic saline was placed in the opened thoracic cavity in quantities sufficient to submerge the heart when a pair of distant recording electrodes was used.

c. A lead plate was interposed between the heart and the posterior paravertebral muscle mass when a pair of distant recording electrodes was used.

d. A metal oncometer made of tin foil or of lead was placed around the ventricles and in contact with the heart and posterior paravertebral muscle mass when a pair of distant recording electrodes was used. In most experiments this oncometer was used when the chest was filled with saline.

e. Mercury, 10 to 30 cc., was introduced into the pericardial sac by means of a pericardial fistula as described by Katz and Gauchat.

Discussion of results. The application of a gauze pad around a recording electrode located on an uninjured area of the heart led to a diminution in the amplitude of all the electrical deflections. This was true whether the second recording electrode was on another uninjured area of the heart (cf. fig. 1) or at a distant point away from the heart. This effect was due, in part at least, to the gauze pad offering a better by-pass for the currents generated by the heart than existed before, so that less current passed through the galvanometer circuit. The minor changes in contour, caused by the application of a gauze pad on the ventricles when distant recording electrodes were employed, are ascribed to this action also. However, the effect shown in experiments such as that illustrated in figure 1 may be caused in part by the increase in the area of the heart from which electric currents are picked up. Since events in a larger area are apt to be more out of phase than in a smaller one, the electrical currents will tend to neutralize each other more by way of the shunt in a large contact electrode than in a smaller one.

The importance of this latter action is brought out when the gauze pad is placed around a recording electrode on a small injured area, the other recording electrode being either on an uninjured region of the heart or at a distance from the heart. The larger contact electrode produced in this

way brings in the currents of the less injured and of the uninjured regions surrounding the injured area. Because the former two areas are larger in extent than the injured one, they come to dominate the recorded electrical curve and so change the configuration of the record from the more or less

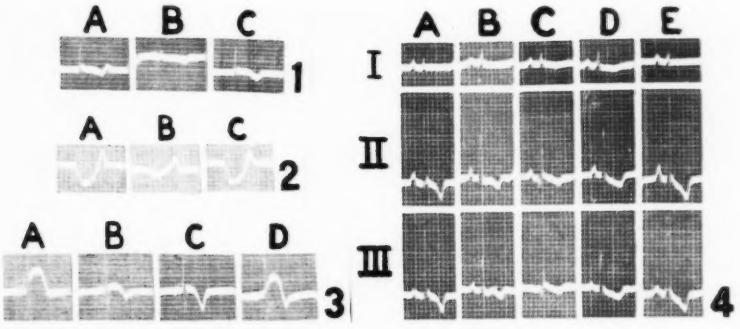

Fig. 1. Electrograms obtained from recording electrodes placed directly on two uninjured regions of the heart. Segments A and C taken without and segment B with a gauze pad soaked in isotonic saline placed around one of the recording electrodes. Discussed in text.

Fig. 2. Electrogram obtained from two recording electrodes, one placed directly on an injured region of heart and connected to the right arm terminal of the electrocardiograph, the other at a distance from the heart (diaphragm) and connected to the left arm terminal. Segments A and C taken without and segment B with a gauze pad soaked in isotonic saline placed around the electrode on the heart. Discussed in text.

Fig. 3. Electrograms obtained from two recording electrodes, one placed directly on an injured region of heart and connected to the left arm terminal of the electrocardiograph, the other at a distance from the heart (diaphragm) and connected to the right arm terminal. Segments A and D taken without and segments B and C with a gauze pad soaked in isotonic saline placed around the electrode on the heart. The gauze pad used when segment C was recorded was twice as thick as that used when segment B was recorded. Discussed in text.

Fig. 4. Electrocardiograms (standard three limb leads) showing the effect of putting isotonic saline in the chest cavity and the effect of surrounding the heart with a metal oncometer on the magnitude and contour of the curves. Segments A and E are controls. Segments B and D taken after the open chest was filled with warm isotonic saline. Segment C taken after a metal oncometer had been placed around the heart, in contact with it and the posterior paravertebral muscle mass. Discussed in text.

monophasic type to a more nearly diphasic form as is illustrated in figures 2 and 3. The alteration in contour produced by this simple procedure resembles the alterations observed in serial records taken during the disappearance of injury currents from the heart (Korey and Katz). Thus,

in figure 2, and especially in figure 3, a final deflection, opposite in direction to the deviation of the mid-portion of the curve, appears or becomes larger, while the deviation of the mid-portion diminishes. The initial oscillations also decrease in size. In most instances the diminution of the initial oscillations was quite marked as in figure 2. In only two instances, of which figure 3 is one example, did the initial oscillations remain practically unchanged. In both of these the second electrode was at a distance from the heart. Apparently, in these instances, the shunting was better for the injury currents than for the ordinary action currents.

The introduction of warm saline in the chest in most instances also caused a decrease in the amplitude of the deflections in the standard three leads, cf. figure 4 or 5. This diminution is also the result of shunting of

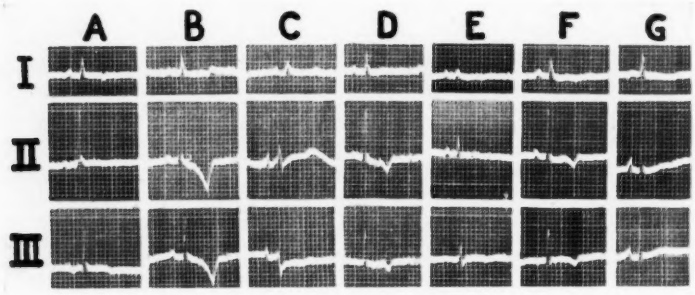

Fig. 5. Electrocardiograms (standard three limb leads) showing the effect of putting isotonic saline in the chest cavity and the effect of surrounding the heart with a metal oncometer on the magnitude and contour of the curves. Segments A and G are controls. Segments B, D, and F taken after open chest was filled with warm isotonic saline. Segments C and E taken after metal oncometer had been placed around the heart, in contact with it and the posterior paravertebral muscle mass. Discussed in text.

the heart currents by paths other than that through the galvanometer. Sometimes, when saline was first introduced, presumably because its temperature was below that of the heart, temporary alterations in the electrocardiograms were seen which tended to mask the diminution of the amplitude of the waves (cf. segments A and B of fig. 5). In later records this tended to disappear as the saline became warmer, (cf. segments B, D and F of fig. 5). In addition to these temporary changes, permanent alterations in the contour of the T wave and in the relative height of the QRS complex in the three leads were seen. These are doubtlessly due to alterations in the electrical field which follow the increase in area of the heart from which currents are carried away by the saline bath and the modifica-

tion of the paths by which the electric currents generated by the heart spread through the body.

The factors outlined above as operating in the saline bath experiments also operate in the experiments where metal plates and metal oncometers were introduced. In the latter experiments the shunting effect exerted the dominant rôle, however, because of the great disparity in the conductivity of the metal and body tissues. This led to a great diminution in the amplitude of the deflections in practically all experiments following the introduction of the metal oncometer or plate, viz., figures 4 and 5. Changes in contour were also present, the cause of which is probably the same as given above to explain similar changes following the introduction of saline in the chest.

It is noteworthy to emphasize that an identical type of results can be obtained by introducing around the ventricles or between the ventricles and the posterior paravertebral muscle mass either an electrical insulator or

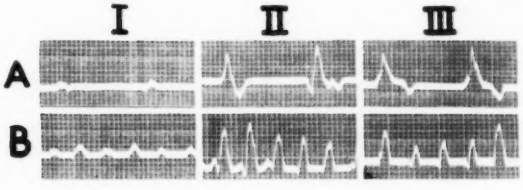

Fig. 6. Records obtained from standard three limb leads in a dog dead a week, showing the effect of dabbing a mercury filled lung on the posterior wall of the open chest. Rate of dabbing in segment A, about 80 per minute; in segment B, about 300 per minute. Discussed in text.

a metal conductor which is greatly superior to the body tissues as an electrical conductor. (Compare the above results with those reported by Katz and Korey.) In the former case the currents generated by the heart are more or less confined to the region between the heart and the insulator; in the latter, the currents are more or less prevented from passing the metal conductor which shunts them back to the heart.

Mercury in the pericardial sac led in our experiments to the same bizarre artifacts which have been previously reported from this laboratory when mercury was lodged in the heart cavity and in the lung vessels (Buchbinder and Katz). We found that these cyclic changes could be reproduced both in the living and dead dog by dabbing a piece of lung filled with mercury up and down on the posterior wall of the open chest. In figure 6, for example, is shown the effect on the standard three leads of dabbing the mercury-filled lung on the posterior paravertebral muscle mass at the spot where the heart had been located, in a dog dead one week. The deflections

were produced every time the sac came in contact with the muscle mass. This phenomenon is due to electrostatic charges set up by the agitation of the mercury in the lung (frictional electricity) which are discharged through the body of the dog when the lung sac comes in contact with it. While shunting may play a part in the causation of the electrocardiographic distortions with mercury in the living animal, it would seem that the creation of electrostatic charges by friction is much more important. This raises the question as to whether or not frictional electricity plays any rôle in causing the electrocardiographic deflections in the normal animal or man.

SUMMARY AND CONCLUSIONS

1. The introduction of good electrical conductors adjacent to the heart causes alterations in the electrical records obtained from direct, from distant, or a combination of direct and distant electrodes.

2. Evidence is given to suggest that the conductors introduced operate by a, offering a by-pass for the currents generated by the heart, thereby decreasing the amount of current passing through the galvanometer circuit; b, altering the path taken by the currents from the heart to distant points; c, altering the relative contribution of the various regions of the heart to the recorded electrical curves, and d, creating electrical stresses, the result of frictional electricity between dissimilar conductors.

3. The relative importance of these actions depends on a, the manner of recording the electrical curves; b, the presence or absence of regions of injury in the heart, and c, the nature and location of the electrical conductor introduced.

4. These observations reëmphasize and define, in part at least, the importance of the electrical properties of tissues adjacent to the heart in determining the electrical field set up by it.

REFERENCES

Buchbinder, W. C. and L. N. Katz. Am. J. Med. Sci. **187:** 785, 1934.
Jochim, K., L. N. Katz and W. Mayne. This Journal **111:** 177, 1935.
Katz, L. N. and H. W. Gauchat. Arch. Int. Med. **33:** 371, 1924.
Katz, L. N., I. Gutman and F. H. Ocko. This Journal, in press.
Katz, L. N. and H. Korey. This Journal **111:** 83, 1935.
Korey, H. and L. N. Katz. Am. J. Med. Sci. **188:** 387, 1934.

THE UREA CLEARANCE OF RATS: ITS TECHNIQUE AND NORMAL RANGE

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The thoroughness with which the nutrition of the rat has been studied, the availability of the animals, and the low upkeep charges make this animal peculiarly suited for physiological observations. The following method was evolved which permits the study of renal function during metabolic observation of this animal.

Metabolism cage. The ordinary metabolism cages for collecting urine of small animals were found to interfere with certain tests because reactions occurred between urine constituents and the iron funnel. To obviate this difficulty and to secure specimens suitable for microscopic examination, a collection funnel in the shape of a very broad and shallow cone was made of aluminum 1 mm. in thickness. This funnel fitted snugly into the standard battery jar (see fig. 1). A hole about 1 cm. in diameter in the center of the sheet provided an outlet for the urine. To prevent fecal contamination of the urine a single layer of cheese cloth was placed across the outlet of the funnel, and attached to the under surface of the aluminum cone by painting the edges with melted paraffin. The aluminum cone was supported by three wooden pegs about two inches high; over the cone was placed a platform of 10 mm. galvanized iron mesh to support the rat and to allow unobstructed pathways for drainage of the urine. Beneath the outlet of the collection cone a 15 cc. wide mouth bottle was placed, and stuck firmly to the bottom of the jar with plasticine.

The drinking apparatus consisted of a centrifuge tube corked with a 1-hole rubber stopper through which was led a glass tube of 6 mm. external diameter. The lower end, from which the rat drank was contracted to an inner diameter of 4 mm. to prevent dripping. A wire spiral placed in the tube from the lower end to the reservoir prevented stoppage of flow by air bubbles. During clearance periods a graduated centrifuge tube was used to facilitate measurement of the intake.

CONDITIONS FOR UREA CLEARANCES. 1. *Diet during clearance test.* It was found desirable to train the rats for this type of study. When young, about 40 grams in weight, they were put through the routine of urine collection, and trained to take fluids from a glass tube for several weeks

before any tests were attempted. It may be mentioned that rats fed and trained in this manner usually spilled little or none of the milk; thus the urine collected was suitable for microscopic examination for red blood cells, casts, and also for chemical analysis.

It was impractical to feed the animals with their usual solid rations during clearance periods because of food contamination of the urine. When only water was given during this period, the volume of urine voided was small; and this increased the inherent error. The most satisfactory results were obtained when a total of 32 cc. of milk was given in four equal feedings during the 30 hours of the combined two clearance periods. Most rats took the 32 cc. of milk without urging; but to entice the few

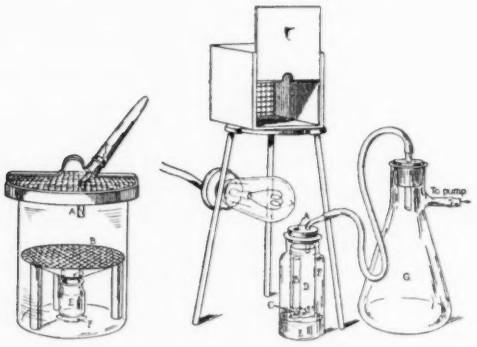

Fig. 1 Fig. 2

Fig. 1. Urine collection jar and feeding tube, *A*; drinking tube, *B*; galvanized iron mesh support for animal, *C*; aluminum collection cone, *D*; gauze strainer, *E*; collection bottle set in plasticine, *F*.

Fig. 2. Apparatus for bleeding, showing box for holding animal with lamp beneath for warming and tube for collecting blood samples. *A*, tapered hole for animal's tail; *B*, oxalated tube for blood; *C*, cotton; *D*, supporting glass tube set in plasticine, *E*; *F*, suction bottle; *G*, trap.

who had no such avid appetite for milk, 1 cc. of cod liver oil was added. This usually induced consumption of the 32 cc. A few animals refused to take this amount of fluid; and the results obtained with them were difficult of interpretation; hence it seems best to use only animals receptive to training. Despite the apparent large volume of food intake, some rats can consume even more. For example, animals weighing only 50 grams, have taken as much as 40 cc. in the 30-hour period when it was offered. Such large intakes were occasionally followed by diarrhea in young animals.

2. *Urine collection and time of periods.* During the six hours before the clearance was begun, the animals were fed 6 cc. of the milk mixture. At the end of this time the bladder was compressed to insure emptying and

to minimize errors from residual urine at the beginning of the clearance. Under these conditions any urine in the bladder at the beginning of the test period was of approximately the same urea concentration as that excreted during the test. This prevented elevation of first period clearance by contamination of clearance specimens with highly concentrated preclearance urine. The urea clearances were done over two periods of time totalling about thirty hours. The animals were usually placed on the urine collection apparatus about 5 p.m. The first period ended about 10 a.m. the following morning, when the bladder was completely emptied by compression and the urine was taken from the collection bottle for measurement of volume and analysis. Immediately thereafter the rat was weighed and bled; then returned to the collection jar for the second period, which usually terminated about 10 p.m., when the bladder was again compressed to insure emptying. Shorter periods may be used if the animal is voiding freely. The urine volumes were measured by drawing the urine from the collection bottle into graduated pipettes.

3. *Blood collection.* In our hands venepuncture was not a reliable means of obtaining repeatedly the requisite amounts of blood. Simple clipping of the tail was likewise unsatisfactory; for unless the tail was clipped so close to the body as to render it unfit for repeated bleedings, the blood flowed so slowly that clotting occurred. These difficulties were obviated by applying moderate suction to the rat's tail, with only the tip removed.

The animal was placed in a small wooden box screened at one end with wire mesh and closed at the other with a sliding door, which was notched to permit passage of the tail (see fig. 2). The tail was immobilized and at the same time a tourniquet action was effected by applying a strip of adhesive tape as follows. The mid-portion of an adhesive strip, 6 × 100 mm. in area, was placed over the root of the tail where it projected through the door. The right end of the strip was pulled down, crossed under the tail and attached to the left side of the box. The opposite maneuver was effected with the left end of the strip after it had been pulled snugly about the tail. The box with the rat was placed on a ring stand, and warmed with a 100 watt lamp placed below it.

For the first bleeding, approximately 3 mm. of tail was removed with a sharp scalpel or razor. The cut end of the tail was inserted into a wide-mouth suction bottle (see fig. 2), having a capacity of about 100 cc. and a height of 11 cm. This was fitted with a 2-hole rubber stopper, with one hole tapered to fit the tail. When the tail was inserted into the hole, vaselin was applied with a syringe about the tail where it entered the cork, so that an air-tight fit of the tail was obtained. Through the other hole in the stopper was led a 6 mm. glass tube which was attached to a vacuum system. The tail was passed through the stopper into a small glass tube 1 × 6.5 mm. which had previously been coated inside with potassium

oxalate. This tube was kept in position by inserting it into a larger tube of about 2.5 × 9.5 cm. dimensions, which fitted the suction bottle as shown in figure 2. The larger tube was immobilized by fixing it to the floor of the suction bottle with plasticine or sealing wax. Cotton was placed in this tube to provide a soft receptable for the oxalate tube and thereby prevent breakage. Because the amount of vacuum obtained by mouth suction was insufficient for bleeding purposes, a suction pump was used. The tail was sometimes sucked into the cork with such force as to cut off arterial circulation. Whenever this happened the tail was partially withdrawn from the stopper to allow reëstablishment of the circulation. During collection of blood the suction chamber was gently agitated to inhibit formation of minute clots. At the termination of bleeding, hemorrhage was stopped by the application of collodion. Healing resulted in a somewhat bulbous tip, of such vascularity that on subsequent bleedings it was necessary to remove only about 1 mm. To recapitulate: Successful bleedings depend upon three factors: 1, tourniquet effect; 2, peripheral dilatation by warming, and 3, suction, each of which was indispensable. When the procedure was followed, as described, 2 to 3 cc. of blood could be easily obtained.

The oxalated tubes were prepared by distributing 0.1 cc. of neutral 20 per cent potassium oxalate solution in a film about the wall of each tube. The tubes were then placed in a desiccator with calcium chloride as drying agent. The desiccator was kept evacuated until all traces of moisture had disappeared from the tubes, about four hours. The thin layer of crystalline oxalate in the tube dissolved instantly on contact with blood, and prevented clotting while the blood was running down the side of the tube.

Urea determinations. The blood urea was determined by the micro hypobromite method described by Farr (1935), which allows duplicate analyses to be done on 0.2 to 0.25 cc. of whole oxalated blood. Urine urea was determined by the hypobromite method of Van Slyke (1929, 1933). Urease urine determinations as described by Van Slyke (1927) may be used, but, as pointed out by Van Slyke, Page, Hiller, and Kirk (1935), the combined excretion of ammonia and urea N, obtained by the hypobromite method, gives somewhat more consistent clearance values than the excretion of urea alone, which is obtained by the urease method.

Error in collection and analysis of urine. Recovery trials in which a known volume of water has been poured into the cage indicate a maximum loss of 0.4 cc. and a minimum loss of 0.2 cc. The largest volume of urine that the rat probably could retain in the bladder without detection may be estimated at 0.5 cc. Hence the maximum error in volume measurement may be expected to approximate 1 cc. The usual error will of course be considerably under this figure but it is evident that maximum urine

volumes, preferably 10 cc. or over, are essential in order to limit effects on the clearance caused by error in urine volume.

The error caused by incomplete emptying of the bladder at the beginning of the first period would be magnified if the retained urine were of markedly greater concentration than the urine excreted during the period. The preliminary period of fluid administration minimizes the probability of such concentrations. Under the feeding régime used any residual urine will probably approximate the same urea concentration as the clearance urine, and the error introduced is limited chiefly to the volume measurement. If the preliminary feeding period is not used the error that may be introduced is very significant, as under ordinary conditions urea nitrogen concentration in rat urine will often run well over 2 per cent Errors introduced by the factors above discussed can usually be detected by wide discrepancies between the clearances for the two periods. When such evidence of probable error is seen, it is desirable to repeat the clearance test.

Calculations. The blood sample for urea determination was taken at the end of the first test period, and was assumed to represent an average value for the entire test collection period. This allowed the measurement of two clearances over two successive periods, with one blood urea determination.

The clearances were calculated according to Møller, McIntosh, and Van Slyke (1928), as cubic centimeters of blood cleared of urea per square meter of surface area per minute. The surface area was calculated according to Meeh's formula (1879), $A = 9.1 \sqrt[3]{W^2}$, where A is the surface area and W the body weight of the rat and 9.1 is the characteristic constant for rats as calculated by Rubner (1902).

The formula used for calculating surface area may not be equally exact for all weight groups, and thereby an error may be introduced into the clearance calculations. This error could, however, hardly be significant, in view of the usual width of the clearance range in an individual.

Results. *Clearance range of group of normal rats.* The results of 59 clearances on 8 normal rats are summarized in table 1. Complete data on one rat are shown in table 2. The distribution of these clearances is shown in figure 3, which it will be noted approximates a curve of the usual probability type. The mean average clearance was 10.9 cc. per square meter of body surface per minute. The standard deviation was $\pm$ 3.1 cc.

Data are insufficient to establish whether or not a rat has an augmentation limit.

Six rats in this group were subsequently killed and their kidneys examined microscopically; no significant pathological renal changes were detected.

Clearance range of an individual rat. These studies indicate that it is

difficult to evaluate definitely the usual state of a rat's renal function from a single urea clearance. However, a series of observations permits of a definite interpretation. Thus the initial clearance on rat 4 on 5/25 of

TABLE 1

Summary of clearances and blood urea determination on 8 normal rats

RAT NUMBER	DATE	BLOOD UREA NITROGEN	UREA CLEARANCE	RAT NUMBER	DATE	BLOOD UREA NITROGEN	UREA CLEARANCE
		mgm. per cent	*cc./sq.m./ min.*			*mgm. per cent*	*cc./sq.m./ min.*
1	5/17	14.5	10.8	4	5/25	29.8	4.6
	5/22	15.5	12.5		6/7	32.8	9.7
	5/25	4.7	43.8		8/7	13.7	9.5
	6/3	17.0	9.2		8/21	18.1	8.0
	8/6	11.9	9.0		9/5	18.0	8.0
	8/21	11.3	10.5		10/11	16.0	5.9
	9/5	12.8	10.0		11/15	15.7	12.5
	10/11	21.0	11.3				
	11/26	28.0	10.2	5	5/26	25.3	6.8
					6/7	9.0	10.3
2	5/17	18.2	14.6		8/7	13.9	7.4
	5/22	19.0	12.5		8/21	15.9	12.7
	5/25	13.5	14.3		9/5	21.3	7.2
	6/3	19.7	11.2		10/11	16.4	7.2
	8/6	13.0	10.9		11/26	22.3	9.0
	8/21	21.3	5.8				
	9/5	16.8	14.0	6	5/26	32.3	8.7
	10/11	15.8	14.8		6/7	15.5	6.4
	11/29	17.8	10.3		8/7	21.4	8.7
					8/21	21.0	12.8
3	5/17	21.4	11.9		9/5	18.8	12.1
	5/22	47.3	5.6*		10/11	21.0	15.6
	5/25	20.1	9.7		11/29	21.1	11.6
	6/3	7.3	17.9				
	6/7	8.7	15.0	7	5/17	12.4	17.4
	8/7	13.4	10.1		5/22	28.0	9.1
	8/21	15.3	14.2		5/25	24.6	8.9
	9/5	12.5	14.3		6/3	17.7	13.3
	10/11	14.0	16.6				
	11/29	22.0	7.8	8	5/17	28.6	13.5
	12/5	16.9	8.0		5/22	24.1	7.5
	12/15	18.0	17.8		5/25	20.1	10.6
	1/9	23.5	11.2		6/3	18.6	7.3
	1/21	20.7	11.7				

*Rat was ill.

4.6 cc./sq. m./min. would indicate an impairment of function, yet the mean average for this animal was 8.3 cc./sq. m./min. which is well within the normal range. Another example of a value which is misleading when

TABLE 2

Detail of urea clearances and calculation on rat 3

DATE	WEIGHT	SURFACE AREA	URINE N PER 100 CC.	BLOOD UREA N PER 100 CC.	TOTAL FLUID INTAKE	TOTAL URINE VOLUME	TIME	VOLUME OF URINE PER MINUTE	U/B	$\frac{UB}{V}$ OBSERVED CLEARANCE	$\frac{UV}{BA}$ CLEARNCE PER m^2	AVERAGE CLEARANCE PER m^2
	grams	*square meters*	*mgm.*	*mgm.*	*cc.*	*cc.*	*minutes*	*cc.*		*cc.*	*cc.*	*cc.*
5/17	60.0	0.0139	349.0	21.4	22.0	11.8	1,135	0.0104	16.3	0.170	12.1	
			174.2		16.0	9.1	498	0.0183	8.15	0.149	11.7	11.9
5/22	77.0	0.0164	327.0	47.3	16.7	9.9	1,112	0.0089	6.9	0.062	3.7	
			820.0		8.5	3.5	457	0.0074	17.4	0.129	7.9	5.6*
5/25	78.0	0.0166	483.6	20.1	12.3	9.7	1,081	0.0090	24.0	0.215	12.9	
			181.0		10.0	6.7	589	0.0120	9.0	0.108	6.5	9 7
6/3	104	0.0201	162.0	7.3	13.0	7.8	488	0.0160	22.5	0.360	17.9	17.9
6/7	112	0.0211	140.1	8.7	13.0	9.7	494	0.0196	16.2	0.318	15.0	15.0
8/7	230	0.0342	331.5	13.4	16.0	16.8	1,017	0.0165	24.7	0.408	11.9	
			287.2		16.0	9.9	757	0.0131	21.4	0.279	8.2	10.1
8/21	249	0.0360	573.0	15.3	9.9	9.5	1,100	0.0086	37.5	0.323	9.0	
			864.0		16.0	8.4	678	0.0124	56.5	0.699	19.4	14.2
9/5	268	0.0378	475.0	12.5	7.2	14.9	1,088	0.0137	38.0	0.523	13.8	
			649.5		16.0	5.9	543	0.0109	51.9	0.562	14.9	14.3
10/11	287	0.0396	963.6	14.0	16.0	10.7	1,111	0.0096	68.9	0.664	16.8	
			532.0		12.4	8.2	482	0.0170	38.1	0.648	16.4	16.6
11/29	328	0.0433	980.0	22.0	16.8	8.8	1,040	0.0085	44.5	0.376	8.7	
			900.0		8.0	5.0	685	0.0073	40.9	0.299	6.9	7.8
12/5	330	0.0435	619.0	16.9	19.0	12.6	1,094	0.0115	36.7	0.422	9.8	
			756.0		13.0	4.6	662	0.0070	45.2	0.315	7.3	8.0
12/15	331	0.0435	621.3	18.0	10.0	9.7	313	0.0310	34.5	1.068	24.5	
			549.6		10.0	11.3	720	0.0157	30.5	0.480	11.0	17.8
1/9	347	0.0449	710.6	23.5	26.0	18.2	1,080	0.0167	30.4	0.508	11.3	
			913.0		12.0	8.1	640	0.0127	38.8	0.492	11.0	11.2
1/21	347	0.0449	867.0	20.7	25.0	15.3	1,090	0.0140	41.8	0.599	13.4	
			930.0		12.0	5.7	570	0.0100	44.9	0.449	10.0	11.7

* Rat was ill.

isolated is the clearance of 5.8 cc./sq. m./min. on 8/21 on rat 2. This animal consistently showed a clearance close to the mean average of 10.9 cc./sq. m./min. In general, three clearance determinations gave a fairly accurate picture of the renal function. Very few animals showed a clearance range as great as that shown by the entire group, and most rats seemed to approximate a characteristic individual mean, which might be considerably above or below the mean of the group. Exceptional clearance values, markedly outside the usual range, occur occasionally from the influence of factors at present unknown. Such a clearance is that one on 5/25 on rat 1 which was 43.8 cc./sq. m./min. Other rats studied but not included in this series have infrequently shown clearances of this magnitude. These exceedingly high values are not characteristic and are not used in estimating the animal's range. Whether such clearances actually occur, or were due to unnoted errors, is uncertain. They became more rare as our experience increased.

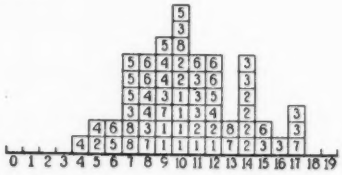

Fig. 3. The distribution of 59 urea clearances on 8 normal rats. The figures within the squares identify the animal. The abscissae represent urea clearance in cubic centimeters per square meter of body surface per minute. Mean, 10.9 cc. standard deviation ±3.1 cc.

Temporary clearance depressions not associated with renal damage. In rats (as one of the authors (L. E. F.) has also noticed in human infants) any illness associated with prostration may be accompanied by a temporary fall in renal function. Such a temporary depression of renal function, with resultant azotemia, due primarily to extra-renal cause, is illustrated by the clearance on rat 3 on 5/22 when the animal had diarrhea. This apparent lowering of renal function was not due to destruction of nephrons, as subsequent clearances were all normal. It is likely that such lowerings of clearance are due to a decrease in renal blood flow. Van Slyke, Rhoads, Hiller and Alving (1934a, 1934b) have shown that renal blood flow in dogs parallels simultaneous variations in clearance, whether the latter were spontaneous or were induced by variations in protein intake.

SUMMARY

1. A method is described for determining urea clearances of rats.

2. Urea clearances on normal rats, on a milk diet, determined in the manner described, showed a mean average of 10.9 cc. per square meter of body surface per minute. The standard deviation was ± 3.1 cc.

REFERENCES

FARR, L. E. J. Clin. Invest. **14:** 911, 1935.

MEEH, K. Ztschr. f. Biol. **15:** 425, 1879. (See also G. LUSK, The science of nutrition. 4th ed. Saunders, 1928, p. 122.)

RUBNER, M. Die Gesetze des Energieverbrauchs bei der Ernährung Deuticke. Leipzig and Wien, 1902, p. 281. (See also G. LUSK, The science of nutrition. 4th ed. Saunders, 1928, p. 122.)

VAN SLYKE, D. D. J. Biol. Chem. **73:** 695, 1927. (See also PETERS AND VAN SLYKE, Quantitative clinical chemistry. Vol. II, Williams & Wilkins, Baltimore, 1932, p. 360.)

J. Biol. Chem. **83:** 449, 1929. (See also PETERS AND VAN SLYKE, Quantitative clinical chemistry. Vol. II, Williams & Wilkins, Baltimore, 1932, p. 360.)

VAN SLYKE, D. D. AND V. H. KUGEL. J. Biol. Chem. **102:** 489, 1933.

VAN SLYKE, D. D., I. H. PAGE, A. HILLER AND E. KIRK. J. Clin. Invest. **14:** 901, 1935.

VAN SLYKE, D. D., C. P. RHOADS, A. HILLER AND A. S. ALVING. This Journal **109:** 336, 1934a.

This Journal **110:** 387, 1934b.

THE EFFECTS UPON THE HEART RHYTHM OF PREMATURE STIMULI APPLIED TO THE PACEMAKER AND TO THE ATRIUM

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The automatic rhythmicity of the pacemaker of the vertebrate heart has rightly been the subject of many investigations. Most, if not all of these investigations have involved the use of records obtained after conduction from the pacemaker to some more or less remote point from which electrical or mechanical records were obtained. The recent papers of Eccles and Hoff (1934) report an extensive study based on the method of premature stimulation of the pacemaker in the cat heart under methods of control which presumably reduced complications in interpreting experimental results due to variations in conduction times. The importance of the conclusions reached by Eccles and Hoff in these papers and the application of their conclusions to the interpretation of factors involved in the rhythmic activities of the heart and its pacemaker seemed to warrant an attempt to repeat their experiments on the turtle heart.

The turtle heart offers an excellent preparation on which to perform such experiments as the sinus region of the heart is easily accessible, the rate of the heart is sufficiently slow so that stimulation may be satisfactorily accomplished by a hand key, and the structure of the heart is such that effective stimuli may be applied to the pacemaker in the sinus, to a large part of the sinus without effect on the pacemaker, to the atrium, to the ventricle, or to any desired combination of these. The experiments reported below deal only with the effects 1, of stimulation by an electrode placed on the sinus as close to the pacemaker as possible and resulting in pacemaker stimulation and, 2, of stimulation by an electrode so placed that it might stimulate either pacemaker or atrium or both according to the responsiveness of the parts considered at the moment of stimulation.

METHODS. For all the experiments considered here, electrographic records were made from the atropinised heart of the turtle (P. elegans). In preparing for an experiment, the brain of the turtle was pithed and the plastron and front legs were removed. A small opening was made in the pericardium and two or three drops of atropin sulphate, 0.1 per cent,

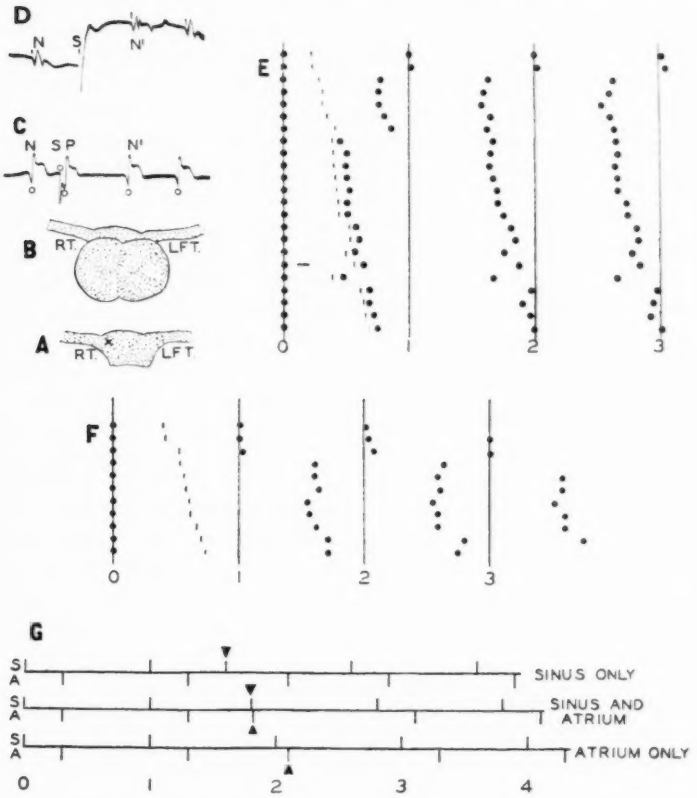

Fig. 1. A. Diagram of the turtle sinus preparation.

B. Diagram of the sinus-atrium preparation.

C. Record made from the sinus-atrium preparation. For purposes of reproduction this record was traced in ink. The record is the one from which measurements for observation number 6, indicated by the short horizontal line, of figure 1-E were made.

D. Redrawn record as obtained from the sinus preparation.

E. Plot of data as measured on a single typical strip of record from the sinus-atrium preparation.

F. Plot of data from a typical strip of record from the sinus preparation.

G. Diagram summarising the types of response obtained in the experiments described. Responses of sinus and of atrium indicated as short vertical lines, respectively, above and below the horizontal lines. Time of stimulation and region of the heart prematurely stimulated by the shock indicated by the arrow heads. For the top line, it is assumed that the stimulus produced a direct effect upon the pacemaker only. For the middle line, it is assumed that pacemaker and atrium are stimulated simultaneously. For the bottom line, only the atrium is assumed to be stimulated.

were injected into the pericardial sac. A few minutes later, the pericardium was opened widely and the ventricle was cut away.

For the experiments first to be described below, the atria were also cut away leaving only the sinus region of the heart *in situ*. The preparation was thus reduced to one which is diagrammed in figure 1-A. The assumed position of the pacemaker for the preparation from which the data for figure 1-F were obtained is indicated by the position of the cross in figure 1-A. We refer to the *assumed* position of the pacemaker because from many attempts to record electrograms from the turtle heart with one lead-off electrode at the site of the pacemaker, there have resulted only a few which seemed to indicate that this localisation may have been satisfactorily accomplished. We believe that we can usually place an electrode within two or three millimeters of the pacemaker. It is probable that we frequently achieve a better localisation than this, but throughout this paper it is to be understood that in no case are we certain of the exact location of the pacemaker.

The pacemaker location indicated by the cross of figure 1-A is slightly more lateral than usual. In occasional instances the pacemaker is found medially at the posterior (lower) border of the sinus. This is not the normal position of the pacemaker and in occasional preparations the pacemaker can be seen to shift from the normal right antero-lateral position to the median position under the effect of continued moderate stimulation of the right vagus nerve.

For the purposes of this paper, experiments were performed on the turtle sinus preparation in the following manner. All electrodes except the indifferent lead electrode to the amplifier grid were of soft silver wire, 18 gauge, with the tip fused to form a small ball. These electrodes were firmly held in an adjustable clamp and were placed so that they touched very lightly against the sinus tissue. The negative electrode for the stimulating condenser discharge (0.25 mfd. through 10,000 ohms series resistance) was placed over the pacemaker. One or two millimeters anterior to this was placed the lead-off electrode to the grounded side of a battery coupled amplifier activating a General Electric supersensitive oscillograph galvanometer. One or two millimeters from the ground electrode was placed the electrode from the positive side of the stimulating condenser. A small artery clip serving as an indifferent lead to the amplifier grid was attached near the middle of the pericardial chamber. The system as used gave a recorded sensitivity of 13 to 20 mm. per mv. By keeping excess fluid from accumulating over the sinus we obtained a recorded potential with each sinus beat of about a quarter of a millivolt.

The anode of the stimulator was then moved about until the galvanometer showed a minimum of shock escape. An approximate threshold for stimulation was determined and double this voltage was usually taken

as the stimulus strength maintained throughout the ensuing half-hour or hour during which records were being obtained. Experiments performed with shock strengths nearer threshold showed no significant difference in results obtained except for the expected failure to obtain effective stimulation as prematurely as was possible with the stronger shock. In some cases the slight irregularity of time of response of the next normal pacemaker response following stimulation was less conspicuous with the less strong shocks. As the beat interval for the spontaneous rhythm was usually about 1.5 second, it was possible to place stimuli with a sufficient degree of accuracy by depression of a tap key.

Records were taken on bromide paper moving at a speed of 17.5 mm. per second. Since we were interested in pacemaker responses, that is, in the first response recorded from the sinus region, measurements were taken from the first downward movement on the records. These times are indicated by the short vertical lines on figure 1-D. Shock times are plotted as of the first moment of appearance of the shock escape as on the record of figure 1-D.

The second series of records to be reported were performed on turtles with both sinus and atrium *in situ*, but with the ventricle removed. Electrograms were recorded with the grid electrode clipped to the posterior margin of the right atrium. The ground electrode was placed near the middle of the ventral surface of the right atrium. The recording system was adjusted to give an excursion of about 1.5 mm. per mv. The anodal electrode from the stimulator was placed near the pacemaker, not touching the atrium. The cathodal electrode was placed in contact with the pacemaker but at such an angle that the wire also touched and would stimulate the right atrium if the shock was applied during the responsive period of the atrium. For these records (see fig. 1-C) measurements were made at the instant of rising activity under the ground lead, i.e., at the moment of beginning rise of the recorded spike. The shock time was again measured as from the first sign of the escape record.

Results. The results of experiments performed on the sinus preparation are illustrated by use of figure 1-F, which shows the time relationships of the responses obtained on one typical strip of record. The various responses have been so arranged as to bring them into a convenient order. The redrawn record of figure 1-D indicates the type of record used. For figure 1-F, times are indicated, using as units the length of the cycle (or mean of two cycles) preceding the last normal response before application of a stimulus. For each observation, the last normal sinus response is placed in time as a dot on the vertical zero line. Moving horizontally to the right from any zero dot, the moment of stimulation is indicated by the short vertical line. As the record of the resulting sinus response was in most cases lost in the recorded shock escape, no attempt is made to

plot the immediate sinus response to the shock although direct observation of the sinus revealed clearly that such responses occurred. Proceeding farther to the right, the dots indicate the times of the succeeding normal sinus beats.

It will be seen that if a stimulus has no effect in displacing the pacemaker rhythm, all the dots should lie on the vertical lines, 0, 1, 2, and 3. The upper two (or three?) plotted responses show no displacement of the pacemaker rhythm. The stimulus fell in each of these cases so soon after the normal response as to find the pacemaker refractory to the stimulus used. All the other stimuli used in this series of experiments were effective and the results are all plotted. There is some irregularity in the time interval to the normal responses next to follow after the premature response, but in so far as an average result is concerned, one may state that there is no significant change from a normal cycle length in the time from the stimulus to the next normal response.

A series of experiments was next performed upon the sinus-atrium preparation. A typical record of this series is reproduced, redrawn in ink for purposes of reproduction, as figure 1-C. The results measured from a single, typical strip of record are presented diagrammatically as figure 1-E. The moment of application of the shock is indicated by the short vertical lines, the beginning of the local atrial responses by the dots. The last normal atrial response before application of a shock is placed on the zero vertical line.

The lowest series of points represents the course of events when the shock was applied just after the normal discharge of the pacemaker but before conduction to the atrium has resulted in the atrial response. (The sino-atrial interval for this turtle was about 0.4 sec. or 0.3 cycle length.) There is a premature response of the atrium due to the direct stimulation of the atrium by the shock. Since the pacemaker rhythm is not disturbed, the next, normal atrial response occurs after a post-extrasystolic compensatory pause and falls on vertical line 2. The interval from the premature atrial response to the following normal response is, of course, longer than one normal cycle length by an amount approximately equal to the prematurity in time of the premature response with respect to the expected time of the suppressed normal atrial response. A shock applied to the atrium less prematurely would have resulted in a correspondingly lesser degree of lengthening of the succeeding atrial interval.

It is supposed that the shock plotted on the lowest line of figure 1-E was applied at or about the instant of the discharge of the pacemaker in the sinus. As the shock is moved earlier in the cycle, it stimulates both pacemaker and atrium. The time interval from the premature atrial response to the succeeding normal atrial response does not show any further increase but remains essentially constant, being the time of one

normal cycle plus the time for conduction from pacemaker to the atrial lead electrode, minus the time for conduction from the stimulated point on the atrium to the lead electrode.

The last described effects continue with continued shortening of the interval from the last normal response to the stimulus until one reaches a certain critical time, which in this experiment lies at about 0.4 cycle after the last normal atrial response. Numbering upward from the bottom, responses 17 to 21 inclusive do not show a *prompt* atrial response due to stimulation of the atrium by the shock, although there is a delayed premature response of the atrium. This group of responses (17 to 21) is interpreted as due to stimuli falling so early as to find the atrium refractory following its last normal response. The pacemaker is stimulated and gives rise to an impulse which is conducted through the sinus and after a conduction delay results in a response of the atrium. The time required for conduction of this premature impulse is much longer than normal. There is evidence for believing that practically all of the increased conduction time is due to increase in the sino-atrial junctional delay. However, for the present argument it is necessary to point out merely that the time of conduction from pacemaker to atrial lead is longer than normal by a very significant amount. For the following, normal atrial response, the conduction time from pacemaker to atrium is again about normal. Therefore, both the interval from the last normal to premature response and the interval from the premature to the following normal response are shorter than normal. For the two upper lines of figure 1-E, the shock falls so early as to find both the atrium and the pacemaker unresponsive and there is no premature atrial response and no disturbance of the pacemaker rhythm.

Discussion. The results of the two types of experiment outlined above and diagrammed in figure 1-E and 1-F were so easily reproduced as to leave no reasonable doubt as to the validity of the interpretation presented above. Individual differences in the preparations used were expressed in the results obtained. In one turtle, for example, used as a sinus-atrium preparation, the clip used as the grid lead was so placed as to exert considerable pull on the atrium. Because of this, or some other reason, the sino-atrial delay was somewhat over 0.7 second instead of the usual 0.3 to 0.4 second. The atrial records obtained showed a change from the usual result precisely as would have been predicted under such a relationship between sinus and atrial response times. When the atria were cut away and the electrodes were placed on the sinus, the records obtained yielded measurements showing no significant difference from normal.

For the turtle heart, therefore, the results of our experiments on premature stimulation of the pacemaker as recorded from the sinus preparation and of atrium and sinus stimulated separately or together and recorded

from the atrium seem entirely in accord with expectation if one expects a normal pacemaker response to follow a premature response by one normal interval. We have diagrammed in figure 1-G the three major types of result which we have obtained in these experiments. The numbers below the diagram indicate times as expressed in cycle lengths. The temporal position of the shock and the part of the heart stimulated is indicated in each case by the arrow heads. Pacemaker responses (S) are indicated by the short vertical lines above and atrial responses (A) are indicated by the short vertical lines below each horizontal line. For the first case plotted, along the lowest line, the stimulus falls at such a time as to effect a response of the atrium only. There is no displacement of the pacemaker rhythm. On the second line, the stimulus is plotted as falling somewhat earlier, the stimulating electrode touches (or affects) both pacemaker and atrium. There is a prompt premature response of the atrium *and* of the pacemaker. The premature atrial response occurs as plotted. The pacemaker rhythm is displaced and the next atrial response occurs at one cycle plus one sino-atrial conduction interval later. For the uppermost line, the stimulus is considered to fall so early as to find the atrium unresponsive. The pacemaker is stimulated. A conducted impulse reaches the atrium 0.5 cycle later. In both the upper lines, it is considered that when the pacemaker is stimulated prematurely, its next spontaneous discharge occurs at a time one normal cycle length later. Our results thus show a combination of pacemaker and of atrial effects such as that observed in the experiments of Cushny and Matthews (1897) with the mammalian heart. The turtle heart offers a more diagrammatic preparation because its anatomical relationships and the long sino-atrial delay combine to allow a clearer recognition of the locality in which there occur the primary effects of a given premature stimulus.

Such conclusions are obviously at variance with those reached by Eccles and Hoff from their experiments on the cat heart. However, from our experiments and from their published data, we are led directly to the belief that what they consider as *late premature beats* of the pacemaker confuse the two types of result diagrammed along the two lower lines of figure 1-G. On the other hand it seems probable that what they describe as *early premature beats* of the pacemaker involve two other types of response. These responses are in part perhaps simple premature responses of the pacemaker uncomplicated by an immediate atrial response and of the type diagrammed along the uppermost line of figure 1-G. For the other part they appear to be simple premature atrial beats with no immediate effect upon the pacemaker, and with the premature atrial beat falling so early that the next normal atrial beat (the "early subsequent beat" of their fig. 1, p. 354) appears exactly one normal cycle interval after the last normal atrial beat. This situation may be observed in the turtle

heart with a rather strong shock applied through stimulating electrodes so placed that they touch the atrium but not the sinus.

Certain features of our experimental results not considered above in detail seem worthy of note in passing. It is an annoying fact that the pacemaker of the turtle heart is not firmly fixed in its location. It seems possible that one reason for the general failure of accurate localisation of the pacemaker as we have attempted it in our experiments is due to this fact. An attempt to firmly localise a lead-off electrode by pressure, by clipping to the sinus, or by stitching into the sinus wall apparently results in a migration of the pacemaker to some other point. On the other hand, we believe that with the very light contact pressure used in these experiments, the pacemaker usually remained unchanged in position throughout an experiment. In some cases, a sudden change in form of the electrogram indicated that this was not so, although usually after a few beats the record resumed its original form.

Perhaps because of the presence of the electrodes over the pacemaker, there was usually a slight irregularity of the recorded rhythms. Because this irregularity was often more pronounced for one, two, or in a few cases, three beats following the application of a shock, it appears that the shock introduced a further cause of irregularity. This irregularity of rhythm appeared either as a lengthening or as a shortening of the cycle and no consistency in the change of direction was found. In several records, stimuli which apparently failed to produce an immediate pacemaker response caused marked delay in the appearance of the next normal response, thus causing a displacement of the pacemaker rhythm. In one instance, this delay amounted to an increase of about 15 per cent of a cycle length. In a smaller number of cases, "ineffective" stimuli have produced a significantly shortened cycle. The theoretical implications of such results are interesting but as we have been unable to reproduce such results at will, we present these apparent results as such, without explanation and without defense.

SUMMARY AND CONCLUSIONS

1. Using electrograms recorded from the sinus and from the atrium of the turtle heart, a study has been made of the effect of premature stimuli upon the rhythm of the heart.

2. If a stimulus is applied to the normal pacemaker in the sinus at such a time as to give rise to an immediate response, the next spontaneous response, as recorded from a point on the sinus close to the pacemaker, occurs at one normal beat interval after this. Subsequent responses follow at normal intervals thereafter.

3. If a stimulating electrode be placed so as to stimulate either atrium or sinus or both, according to the responsiveness of the tissue concerned

at the moment of stimulation, and if lead-off electrodes be placed on the atrium, and if results be considered in terms of the time of the atrial response, it is found that a slightly premature stimulus results in a slight prematurity of atrial response, a slight lengthening of the succeeding cycle because of the compensatory pause, but no displacement of the pacemaker rhythm.

With further prematurity of stimulation, the pacemaker and atrium are stimulated simultaneously. The succeeding interval (as measured from the atrial record) is long but does not show further significant increase as the stimulus is placed earlier.

When the moment of stimulation is brought still earlier in the atrial cycle, the shock finds the atrium refractory but stimulates the pacemaker and causes a corresponding displacement of the rhythm. However, that premature sinus response may be conducted to the atrium thus causing a premature response of the atrium. Since conduction of this impulse in the relatively refractory phases of sinus and atrium occupies a longer time than normal and since the conduction of the normal sinus beat to the atrium occurs in a normal time, both the cycles preceding and following the premature response will be measured as shorter than a normal cycle.

4. If stimulating electrodes are placed to touch the atrium but not the sinus, an early premature atrial beat may be elicited without causing a dropping of the next normal atrial beat which appears in its proper time.

REFERENCES

CUSHNY, A. R. AND S. A. MATTHEWS. J. Physiol. **21:** 213, 1897.
ECCLES, J. C. AND H. E. HOFF. Proc. Roy. Soc. B **115:** 307, 327, 352, 1934.

LACTIC ACID IN REST AND WORK AT HIGH ALTITUDE[1]

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The present paper sets forth blood lactic acid values obtained from four groups of subjects at high altitude: members of our party in Chile, residents of 'Quilcha, Chile (altitude 5.34 km.), members of our party at Leadville, and residents at Leadville.

The Chilean party was divided into two groups. The first group, consisting of six men, was engaged for approximately six weeks setting up a mobile laboratory at Chuquicamata at an altitude of 2.81 km. A regular program of work on a bicycle ergometer, mountain climbing, and long walks was followed during this period. The second party from sea level, four men, then joined the first, and observations were immediately begun.

The concentration of lactic acid was determined both in arterial blood drawn in the morning before rising and in blood drawn from the antecubital vein after measured performances on the bicycle ergometer. There were 16 days of observations at an altitude of 2.81 km., 8 days at 3.66 km., 12 days at 4.70 km., and 3 weeks at 5.34 km. During this last period parties of two spent from 1 to 6 days at 6.14 km., the top camp. Lactic acid was determined in blood obtained in rest at this altitude, but it was not feasible to transport the ergometer for exercise studies. Values in rest were obtained on six residents at 5.34 km., five of whom were laborers at the sulphur mine, 5.70 km.

The Leadville party consisted of eight members who spent from one to two months at 3.0 km. altitude. Three members spent four days on the top of Mt. Elbert, 4.39 km. Resting lactic values only are presented for

[1] From the International High Altitude Expedition to Chile. The Expedition was supported by grants from the Fatigue Laboratory and the Milton Fund, Duke University; Copenhagen University; King's College, Cambridge University; Columbia University; the National Research Council, the Royal Society, London; the Corn Industries Research Foundation; the Rask-Ørsted Fund, Copenhagen; the Josiah Macy Foundation and the American Association for the Advancement of Science. We are also much indebted to the Chile Exploration Company and to its officers in New York and in Chile, to Señores Hipolito and Juan Carrasco of the S. I. A. M. Carrasco, to Messrs. Packard and Bell of the Poderosa Mining Company, to the Ferro-Carril de Antofagasta a Bolivia, and to the many people, officials and private citizens, who facilitated our work in Chile.

the Leadville party. Work values have been previously published (Dill, Edwards, Fölling, et al., 1931). Resting values were obtained on three residents at 3.0 km. altitude. One of them had spent eight months, and two, several years at this altitude. Lactic acid was determined on venous or arterial blood after one hour of rest.

Lactic acid was determined by the method of Friedemann, Cotonio and Schaffer (1927). That the method holds at high altitude was determined by recovery at sea level of lactic acid under an air pressure of 390 mm., equivalent to an altitude of about 5.5 km. Values in this paper are in milli-equivalents of lactic acid per liter of blood, i.e., mgm. per cent divided by 9.

The lactic acid in rest. Since the observations of Araki (1890, 1891), that acid, which he demonstrated to be lactic, appears in the organism in conditions of anoxemia, there have been varying reports concerning the extent to which lactic acid accumulates in rest during oxygen want. Ryffel (1910) detected only a small accumulation in the blood of man after breathing 12 per cent oxygen for four hours. Later Laquer (1919), in a study of blood lactic acid values in men and dogs found that increases of only a few milligrams per cent occur on going to 2.9 km., with no further changes up to 4.56 km. Barcroft (1925) reports that two subjects had a threefold increase on Monta Rosa: at sea level their values were 1.3 and 1.4 m.-eq., at 2.9 km., 1.9 and 2.0 m.-eq., and at 4.50 km., 4.0 and 4.3 m.-eq. Blood was drawn the morning after arrival. Jervell (1928) found no increase in lactic acid until 7.5 per cent oxygen was breathed, the equivalent of 6.10 km. altitude. More recently Bock, Dill and Edwards (1932) found little or no increase in several subjects after breathing for one hour a mixture of oxygen equivalent to an altitude of 6.70 km. Baicenko and Krestownikoff (1933) found that the lactic acid of two subjects varied between 2.2 and 3.9 m.-eq. on 7 of 10 days at 4.20 km. Their values at Leningrad were 1.0 and 1.6 m.-eq.

In table 1 are included summaries of the resting lactic acid values of the Chilean and the Leadville parties. At the 2.81 km. stop of the Chilean party the average, 1.2, suggests a slight rise over the sea-level average, 0.93. Four men, on the morning after arrival at this altitude from sea level, had an average of 1.5, 1.7 being the highest value obtained. After five weeks at this altitude, four men averaged 1.5. After nine weeks, four men averaged 1.0. Two men, however, after two weeks fell from 1.7 and 1.5 to 0.7 and 0.7. No unbroken altitude increase of over 1.05 km. followed this initial one of 2.81 km., which may account for this slight increase not being noted at the upper stations. On the contrary, at the upper stations the averages remain at the sea-level value, even at 6.14 km. Yet at the 2.81 km. station the arterial saturation was diminished by only a few per cent, while at the 6.14 km. station arterial saturations ranged between 55 and 70 per cent.

It is possible that this initial slight rise may be a reflection of acclimatization processes going on, and that it might again be seen if after acclimatization at 2.81 km. one went directly to 6.14 km.

It is obvious, however, that blood lactic acid in resting man is little affected by changes of altitudes compatible with life, and that after a period of time, sufficient presumably for acclimatization, sea-level values are found. This is strikingly illustrated by a comparison of the sea-level values in table 1 with those of the Chilean residents at 5.34 km.

The Leadville averages agree closely with the Chilean averages. Even better agreement might be expected if the conditions of rest for both

TABLE 1

Lactic acid in rest

STATION	ALTITUDE	NUMBER OF DETERMINATIONS	NUMBER OF SUBJECTS	MAXIMUM	MINIMUM	AVERAGE
Chilean Expedition, 1935 (Arterial blood drawn before rising)						
	km.			*m.-eq.*	*m.-eq.*	*m.-eq.*
Sea level	0	7	7	1.1	0.8	0.93
Chuquicamata	2.81	17	10	1.7	0.7	1.20
Ollagüe	3.66	7	7	1.4	0.7	1.01
Montt	4.70	11	10	1.0	0.7	0.87
'Quilcha	5.34	10	10	1.3	0.7	0.97
'Quilcha residents	5.34	6	6	1.1	0.8	0.92
Punta de Cerro	6.14	8	5	1.2	0.9	1.02
Leadville expedition, 1929* (Venous and arterial bloods after 1 hour rest)						
Leadville	3.00	14	8	1.8	0.8	1.23
Leadville residents	3.00	3	3	1.2	1.0	1.10
Mt. Elbert	4.39	3	3	1.3	1.0	1.20

* Previously unpublished data.

parties had been the same. Because of the time elapsed before the laboratory was set up at Leadville, we have no evidence regarding changes which may have occurred there during early acclimatization.

The lactic acid in work. There is little in the literature regarding blood lactic acid values in work at high altitudes. Laquer (1922) found in one subject that bicycle riding for one hour may produce a slightly higher level of lactic acid at 1.56 km. and 2.5 km. than at sea level. Baicenko and Krestownikoff (1933) found that immediately after a climb to 5.63 km. the lactic acid was 15.2 m.-eq. Results of experiments in low-pressure chambers and breathing low oxygen mixtures will be mentioned later.

In the present experiments the subjects worked for ten minutes at a

constant rate on the bicycle ergometer, and blood samples were drawn at the end of exercise from the antecubital vein. Figures 1 and 2 summarize the data. The concentration of lactic acid has been plotted against the kilogram pressure on the wheel. This manner of presentation has been adopted for the reason that metabolic rates in an individual varied little for a given output of work regardless of altitude. The metabolic changes are discussed by Christensen, Edwards, Forbes and Matthews (in press). Points on the figures represent physical performance and, as shown in the key, the altitude of each experiment. A curve representing the individual's lactic acid levels for various grades of work at sea level has been drawn for each individual except Matthews, who has not been available as a subject.

Figure 1 presents the performances of four members acclimatized to 2.81 km. for six weeks before the experiments were commenced. There is no evidence of extra lactic acid accumulation for a given grade of work in these men even at 5.34 km., except for a few points at 3.66 km. on E. H. C. and A. B. K. In all these men there are in many instances smaller increases in lactic acid for a given grade of work at the higher altitudes than had been observed at sea level, especially in the harder grades of work. In two cases, E. H. C., 2.81 km. altitude, 3.0 kgm. load, and W. H. F., 4.70 km. altitude, 2.25 kgm. load, the rise was distinctly less, but this, however, may be related to the fact that work could only be continued for five minutes. We are not disposed to attach much significance to subnormal rises in lactic acid, but it seems highly significant that for a given grade of work anoxemia does not produce a greater than normal increase in lactic acid.

Figure 2 presents the performance of three men who showed distinct tendencies to reach higher levels of lactic acid at 2.81 km. than at sea level. In the case of H. T. E. the experiment was carried out after 37 days at this altitude. No further experiments on the ergometer were performed on this subject. Those on D. B. D. and J. H. T. were begun the day after their arrival from sea level.

At the higher altitudes these two men no longer reached higher levels of lactic acid than at sea level. Even H. T. E., who showed the most pronounced excess lactic acid formation, probably approached his sea-level curve finally, since at the end of the expedition he had the record number of climbs to the top camp, 6.14 km., a climb that every member found exhausting. On a previous expedition, Dill, Edwards, Fölling et al. (1931) this man finally reached his sea-level performance curve after eight weeks at 3.00 km. On this previous expedition, D. B. D. and J. H. T. showed a lag in approaching their sea-level curves. This lag may be related to physical training since all members of the present expedition, H. T. E. excepted, were in excellent physical condition.

The failure to show higher levels of lactic acid at the higher altitudes not only in various intensities of work but also in rest, may be due to the fact that no single rise after the initial one of 2.81 km. exceeded 1.05 km., or it may mean that once acclimatization has begun at lower altitudes, acclimatization to higher altitudes takes place more readily. In any case, sea-level lactic acid values are eventually reached at any altitude, at least

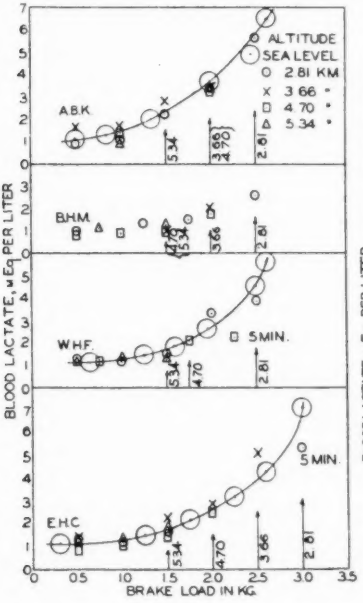

Fig. 1

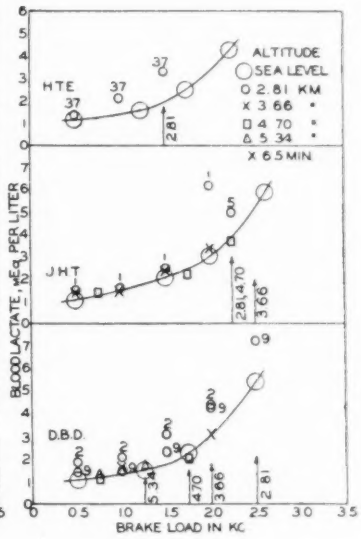

Fig. 2

Fig. 1. Lactic acid levels at various altitudes and grades of work on the ergometer. These four men spent six weeks at 2.81 km. before the experiments were commenced. In this and in figure 2 the arrows indicate the maximum load that each subject could pull for 10 minutes at the altitude designated by the figures beside the arrows, and the line is drawn through observations at sea level.

Fig. 2. Lactic acid levels at various altitudes and grades of work on the ergometer. The numbers above the 2.81 km. points represent days since arrival from sea level.

up to 6.14 km. It is significant that, as in the case of the resting lactic acid, the increases over sea-level values occur at the initial station, 2.81 km., where arterial saturation was diminished only a few per cent, yet there were no increases at upper stations where arterial saturations were as low as 55 per cent.

The arrows on the graphs of figures 1 and 2 indicate the maximum load that each subject could pull for 10 minutes at the designated altitude.

For example, E. H. C., figure 1, could ride with no more than 1.5 kgm. load for 10 minutes at 5.34 km. altitude. The lactic acid accumulated was the same as at sea level for this grade of work, 1.7 m.-eq. Yet at sea level he was capable of riding at the same rate with double the load, accumulating 7.1 m.-eq. of lactic acid. In other words, the ability to perform work is lessened progressively with increase in altitude, and also, with acclimatization, the ability to accumulate lactic acid is lessened. Work experiments of Clark-Kennedy and Owen (1927) performed at sea level while breathing mixtures of low oxygen pressure, led to the conclusion that "the degree of acidosis is proportional not only to the grade of work but inversely to the oxygen tension of the inspired air." The latter part of this statement is completely at variance with our observations on fully acclimatized subjects. Chamber experiments, unless the subjects are in good physical training and have been allowed sufficient time for acclimatization, may be poor substitutes for field studies on men leading an active life.

The inability to produce appreciable amounts of lactic acid at very high altitudes after acclimatization is illustrated in an experiment on D. B. D. and H. T. E. after 5 and 17 days respectively at 5.34 km. After riding mules to 5.70 km. they climbed intermittently for more than an hour, stopping frequently because of exhaustion. On arrival at the top camp, 6.14 km., the lactic acid values were only 1.76 and 1.83 m.-eq. That the values on D. B. D. and H. T. E. are probably maximal is suggested by the paper of Margaria and Edwards (1934) in which they found that in exhausting intermittent exercise of this type the highest attainable lactic acid values are soon reached. Further work is then carried on, as postulated by Margaria, Edwards and Dill (1933) by an alactacid mechanism.

At 6.14 km. altitude arterial saturations were between 55 and 70 per cent. In this range the oxygen dissociation curve is steep. Therefore, a large accumulation of lactic acid would result in a large diminution in the already low arterial saturation. Accordingly, this inability to increase lactic acid seems to reflect a protective mechanism. It is difficult to correlate these findings with the 15.2 m.-eq. of lactic acid found by Baicenko and Krestownikoff (1933) previously mentioned.

That the protective mechanism is very stable after acclimatization is shown by an experiment on E. H. C. The subject after one week at 5.34 km. breathed throughout the experiment sea-level equivalent of oxygen while riding with 3.0 kgm. pressure, a task he had been quite capable of carrying on at sea level for 10 minutes. In 2 minutes and 42 seconds he stopped in an acute state of exhaustion which persisted several minutes. Yet the lactic acid level reached was only 1.67 m.-eq., compared to 7.1 m.-eq. after working for 10 minutes at this rate at sea level. As can be seen from his performance curve, figure 1, the 1.67 m.-eq is almost

identical with the lactic acid for half the load when exhaustion was reached after 10 minutes at this altitude. There was only a slight rise (to 2.01 m.-eq.) after 5½ minutes of recovery.

This diminished ability to accumulate lactic acid and this failure to utilize oxygen reflect marked changes in the organism after acclimatization. Immediately there occurs to one, by way of explanation, the fall in alkali reserve. At sea level, however, when the reserve is diminished to the level at which E. H. C.'s was at 5.34 km., one can still carry on work that reduces the reserve to a much lower value chiefly through the accumulation of lactic acid. In the case of E. H. C. there exists a slight

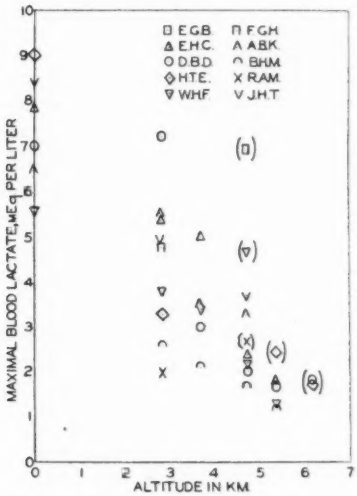

Fig. 3. Lactic acid levels after maximum continuous performance on the ergometer at various altitudes. The points in parentheses represent exhaustion after prolonged intermittent exercise. E. G. B., (□) 4.70 km., collapsed. W. H. F., (▽) 4.70 km., was near collapse.

increase in pHs; in the case of the subject at sea level, a decrease. Bearing in mind, however, the principles of the system as a physico-chemical unit as illustrated by L. J. Henderson (1928) we cannot argue merely from a pH viewpoint.

Figure 3 contains the highest lactic acids attained by each individual at the various altitudes. With increase in altitudes there is a decrease to remarkably low values at 6.14 km. It is obvious from the figure and the explanatory footnote that higher lactic acids can be attained by climbing intermittently to exhaustion than by continuous exercise to exhaustion on the ergometer. This is illustrated by the two points for W. H. F., ▽, (▽),

at 4.70 km. After each stop while climbing intermittently at high altitudes a rapid recovery takes place within a few minutes. This recovery is hardly suggestive of the recovery following a large accumulation of lactic acid in the body. Often one stops with the feeling that it is physically impossible to take another step. Yet, if the legs were the cause of stopping, through accumulation of lactic acid, obviously H. T. E., (◈) 5.34 km., figure 3, and D. B. D., ⊙, and H. T. E., (◈), 6.14 km., figure 3, would have reached much higher levels of lactic acid, since the legs represent such a large portion of the body muscles, and time for the diffusion of the lactic acid was ample. A muscle such as the heart or the diaphragm, however, could become exhausted from the accumulation of lactic acid with little effect on the level of lactic acid in the body as a whole. The intense respiratory difficulty experienced in the region of the diaphragm during the pauses suggests the involvement of the diaphragmatic muscle. It may be that the protective mechanism or the limiting factor in work at high altitudes is the circulatory failure to the diaphragm or the heart.

This leaves unexplained, however, the failure of E. H. C. to utilize the added oxygen, and no explanation is offered here. It may be that the problem is one of utilization rather than of transportation. If so, we become involved in the rôle of myoglobin and the complexity of tissue chemistry. It is not impossible that we are dealing with an enzyme.

It is of interest, in view of the findings in this paper, that Weiss and Ellis (1935) enumerate several of the classical symptoms of exhaustion at high altitudes in cases of cardiac decompensation during work at sea level. Only small amounts of lactic acid could be accumulated. Comparing such performances with those of normals reaching the same low levels of lactic acid, he concludes that "when disturbances in the lactic acid production and peripheral circulation occur, they are the result and not the cause of heart failure."

CONCLUSION

Resting lactic acid values determined on blood drawn in the morning before rising show an initial slight rise over sea-level values on going to high altitudes. Sea-level values are found after acclimatization even at 6.14 km., where arterial saturations range between 55 and 70 per cent.

Standard work performances, on first going to high altitudes, produce greater rises in blood lactic acid than at sea level. After acclimatization lactic acid values similar to those at sea level are found for a given performance. The ability to perform work is lessened progressively with increase in altitude, hence also the ability to accumulate lactic acid. Only slight increases over rest values of lactic acid are found during work at 6.14 km.

The inability to accumulate large amounts of lactic acid at high alti-

tudes suggests a protective mechanism preventing an already low arterial saturation from becoming markedly lower by shift of the O_2 dissociation curve through acid effect. It may be that the protective mechanism lies in an inadequate oxygen supply to essential muscles, e.g., the diaphragm or the heart.

REFERENCES

ARAKI. Ztschr. Physiol. Chem. **15:** 335, 546, 1890.
Ztschr. Physiol. Chem. **16:** 453, 1891.

BAICENKO, I. P. AND A. N. KRESTOWNIKOFF. Arbeitsphysiol. **6:** 359, 1933.

BARCROFT, J. The respiratory function of the blood. Part I. Lessons from high altitudes. Cambridge University Press. 1925.

BOCK, A. V., D. B. DILL AND H. T. EDWARDS. J. Clin. Inv. **11:** 775, 1932.

CHRISTENSEN, E. H., H. T. EDWARDS, W. H. FORBES AND B. H. C. MATTHEWS. (In press.)

CLARK-KENNEDY, A. E. AND T. OWEN. Quart. J. Med. **20:** 383, 1927.

DILL, D. B., H. T. EDWARDS, A. FÖLLING, S. A. OBERG, A. M. PAPPENHEIMER, JR. AND J. H. TALBOTT. J. Physiol. **71:** 47, 1931.

FRIEDEMANN, T. E., M. COTONIO AND P. A. SCHAFFER. J. Biol. Chem. **73:** 335, 1927.

HENDERSON, L. J. Blood; a study in general physiology. Yale Univ. Press, New Haven, 1928.

JERVELL, O. Acta. Med. Scand. Suppl. xxiv, 135, 1928.

LAQUER, F. Ztschr. Biol. **70:** 99, 1919.
Klin. Wchnschr. **1:** 1922.

MARGARIA, R., H. T. EDWARDS AND D. B. DILL. This Journal **106:** 689, 1933.

MARGARIA, R. AND H. T. EDWARDS. This Journal **108:** 341, 1934.

RYFFEL, J. H. J. Physiol. **39:** Proc. xxix, 1910.

WEISS, S. AND L. B. ELLIS. Arch. Int. Med. **55:** 665, 1935.

THE INNOCUOUSNESS OF HISTAMINE TO THE DOG

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The immediate effects of histamine in the animal have been studied extensively (1). The remote or cumulative effects of histamine administration in large amounts over long periods of time have been studied very little. Whether histamine is a poison which will damage the tissues of an animal and so cause one or another derangement of physiological processes has not yet been learned. The popularity of histamine in clinical and laboratory studies makes the data presented in this paper of general interest.

METHODS. A normal young adult non-gravid female dog weighing 10.2 kgm. was attached to the constant-rate intravenous injection apparatus already described (2). A capillary rubber tube (3) was used as the intravenous cannula. After twelve days of preliminary injection of physiological saline solution, during which the animal became accustomed to the experiment, the administration of histamine was begun, and was continued almost without interruption for 62 days thereafter.

The rate of fluid injection was 10 cc. per hour. Histamine was added to the injection fluid (0.8 per cent NaCl) in the amount required for the desired dosages. The histamine used was "Ergamine Acid Phosphate"—Pfanstiehl and "Histamine—Synthetic"—Eastman. All dosages are in terms of the free base, namely, histamine itself.

The dosage of histamine was so regulated as to keep the animal in or near the "sub-retching" state during most of the experiment, in order to administer the largest possible amount of the drug. This dosage varied between 0.066 and 0.7 mgm. per kilogram of body weight per hour, or between 16 and 170 mgm. per 24 hours. The duration of each dosage-level is given by the graph in figure 1.

The animal was allowed the freedom of the cage. Food and water were offered daily. From time to time the blood pressure in the femoral artery was determined by a direct manometric method (4), and blood samples for chemical analysis were taken. The data obtained from these studies appear in table 1.

The injection of fluid was continued for 81 days, but histamine was given for only 62 days. The two interruptions in the injection of histamine

allowed the dog to regain weight and strength lost because of the poor appetite resulting from histamine intoxication. During the course of the experiment the dog received 2,322.1 mgm. of histamine (as free base) and 16,264 cc. of 0.8 per cent sodium chloride solution intravenously.

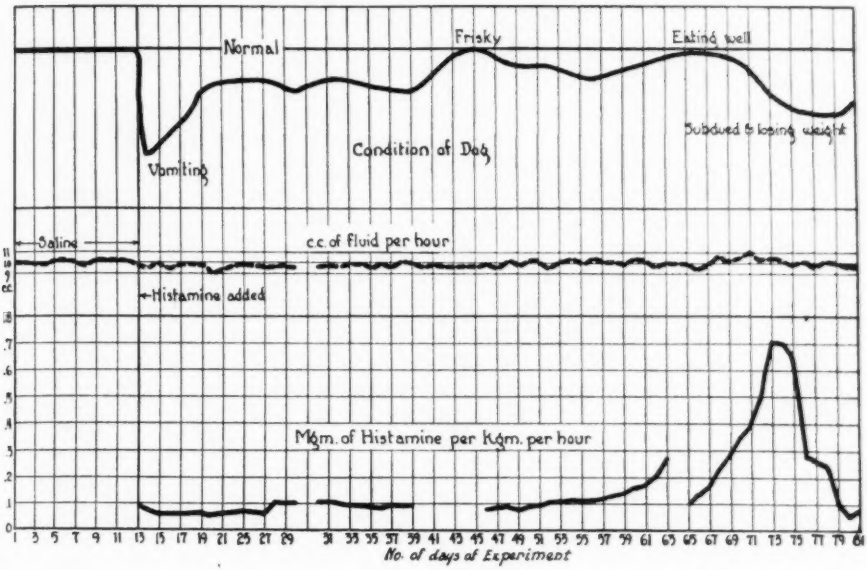

Fig. 1

TABLE 1

DAY OF EXPERIMENT	BLOOD NPN	PLASMA CHLORIDES	BLOOD PRESSURE	BLOOD PLASMA PROTEINS
	mgm. per cent	*mM. per liter*		*grams per 100 cc.*
3	38			
37	30			
44			94	
46	18	80.7		
57	17	104.2	64	5.29
59			74	
71	16	100.0		5.27

Three days after the end of the injection the fresh tissues were examined and specimens were taken for histologic study.

RESULTS. Because there were no measurable phenomena in our experiment by which to gauge the intensity of the effect of the injected histamine,

the general condition of the animal was taken to indicate the degree of intoxication. Our estimate of the general condition of the animal with each dosage-level is presented in the upper curve in figure 1. It is noteworthy that the apparent degree of intoxication for a given dosage does not remain constant, but seems to decrease as the experiment progresses. After many days of histamine intoxication (73rd day of experiment) almost ten times the original dosage caused less prostration than the original dosage (13th day).

Thirst and polyuria were prominent symptoms during most of the experiment. When they became excessive vomiting usually followed. The amount of gastric content lost through vomiting was not large, however. The urine was tested daily for albumen, but it never gave a positive test. The stools, except on two days, were formed and contained no gross blood.

Although the animal was kept in the "sub-retching" state during most of the experiment, it lost weight steadily while histamine was being given. This weight loss was attributed to anorexia caused by histamine intoxication. When the injection of histamine was stopped temporarily (39th to 46th and 63rd to 65th days) the appetite and general condition improved remarkably in a few hours. The dog would then eat large amounts of food and regain weight rapidly. Even at the end of the experiment, when histamine was discontinued for the last time, the animal had lost none of its powers of recovery, but seemed, if anything, the more capable of regaining its weight and strength.

The persistently low non-protein nitrogen content of the blood may in all probability have been the consequence of low food intake and diuresis.

The fresh tissues of the animal were normal in every respect. There were no hemorrhages nor other signs of blood-vessel damage. The gastro-intestinal tract in particular was quite normal. Careful microscopic study of all tissues with especial attention to the vascular structures yielded no signs of degenerative change.

COMMENT. That a normal dog can withstand the effects of chronic severe "intoxication" by histamine without harm supports the idea that this substance is not a powerful protoplasmic poison. On the contrary, all the effects of histamine appear to be completely and easily reversible, and in this sense histamine may be considered innocuous (to the dog).

The apparent acquisition of tolerance indicates the presence of a mechanism by which histamine is metabolized. Although the ultimate fate of intravenously injected histamine is not known, it disappears rapidly from the blood stream, according to Dragstedt and Mead (5) and others (1). It may in part be rendered inert by direct anti-histamine activity observed in dogs' blood by Yen and Chang (6).

SUMMARY

1. A normal dog was given 2,322.1 mgm. of histamine intravenously during 62 days.

2. After this experiment the dog was still normal.

3. There was some evidence of the appearance of tolerance to the drug.

REFERENCES

(1) Best, C. H. and E. W. McHenry. Physiol. Rev. **11**: 371, 1931.
(2) Jacobs, H. R. J. Lab. Clin. Med. **16**: 901, 1931.
(3) Jacobs, H. R. Proc. Soc. Exper. Biol. and Med. **30**: 1160, 1933.
(4) Jensen, C. R. and C. W. Appelbach. Arch. Path. **6**: 99, 1928.
(5) Dragstedt, C. A. and F. B. Mead. Proc. Soc. Exper. Biol. and Med. **32**: 1435, 1935.
(6) Yen, A. C. H. and H. C. Chang. Proc. Soc. Exper. Biol. and Med. **31**: 337, 1933.

REFLEX RESPIRATORY EFFECTS FROM INTERMITTENT STIMULATION OF THE VAGUS AND SUPERIOR LARYNGEAL NERVES

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It is generally agreed that in normal breathing reflexes through the vagi limit the depth of inspiration and at the same time maintain in some way an accelerator influence on the respiratory rhythm. The first of these effects is clearly a reflex from inhibitory afferent fibers, stimulated by inflation of the lungs. The second, or accelerator effect, is still a subject of controversy. In the classical theory of Hering and Breuer, it is attributed to "inspiratory" fibers, stimulated by deflation. Recently Hammouda and Wilson (1932, 1935a, 1935b) have inferred that augmentor fibers are active throughout the respiratory cycle.

There is much evidence indicating that the vagi do contain fibers which, under certain experimental conditions (such as forced deflation of the lungs) have a reflex inspiratory or an accelerator effect. It has not been conclusively shown, however, that these are active in ordinary breathing. Adrian (1933) supports an interpretation suggested by Head (1889). According to this view the inhibitory fibers control directly the depth, and indirectly the frequency of inspiration. When their influence is removed, as by vagotomy, each inspiratory discharge from the center runs its full course, and a long interval of recovery is required before another inspiration is initiated. With the vagi intact each inspiration is cut short through the inflation reflex. Because of the incomplete discharge the center recovers in a comparatively short time and the rhythm is consequently more rapid.

Continuous tetanization of the central vagus, in anesthetized animals, ordinarily causes expiratory standstill. But if Head's hypothesis is correct it should be possible, by intermittent stimulation of the same nerve, to accelerate the respiration after vagotomy. The stimuli should be so timed as to cut short each inspiration at a moderately advanced stage. Under such conditions acceleration would be expected through the indirect action of the inhibitory fibers alone; and even more if augmentor fibers were simultaneously activated.

Several investigators (most recently Gesell and Moyer, 1935) have found that with periodically recurring stimulation the respiration falls into the fixed rhythm of the stimulus. In general, however, little effort seems to have been made, with artificial stimuli, to imitate the normal periodic activity of the inhibitory fibers. Adrian (1933) found that the frequency of impulses (and the number of active fibers) varied with the degree of inflation of the lungs. During inspiration, therefore, the center presumably is subjected to gradually increasing inhibition, from which it is automatically released as the lungs are deflated. Such a mechanism would never subject the center to inhibition of much greater intensity than is required to stop the inspiration in progress. We have not attempted the difficult feat of imitating, artificially, this natural gradation. In our experiments, the vagus was tetanized with shocks of uniform strength and frequency so long as the lungs were inflated beyond a fixed point.

METHODS. Dogs were used, anesthetized with barbital-sodium. The intravenous dose was usually 0.25 to 0.30 gram per kilo, but when the respiration remained irregular it was sometimes necessary to give more. Both vagi were sectioned in the low cervical region, and the central stump of one was drawn into a moist chamber consisting of a slab of bakelite with a longitudinal groove just wide enough to accommodate the nerve. The stimulating electrodes were silver wires lying across the bottom of the groove.

Respiration was recorded by means of a brass tube passed transversely through the thorax just behind the sternum, with perforations opening into the intrapleural space. One end of the tube was closed, the other connected to a tambour. The lever of the tambour carried, in addition to the writing point, a platinum wire with a free end projecting toward a mercury cup below. Through these, the inspiratory down stroke of the tambour made contact in the primary circuit of a Harvard inductorium, arranged for tetanizing stimuli. The vagus was thus stimulated until expiration broke the contact. It was left at rest except when the lungs were inflated beyond a point determined by the level of the mercury cup. For brevity, we shall simply use the term "intermittent stimulation" in referring to this method. A key placed in parallel with the platinum-mercury contact permitted continuous tetanization when desired.

Ordinarily the stimulus was first adjusted to a strength just sufficient, applied continuously for 5 to 10 seconds, to cause expiratory standstill. The same stimulus was then used intermittently.

RESULTS. Figure 1a shows a typical record. Continuous tetanization produces the usual expiratory standstill, but intermittent stimulation causes an increase in frequency with reduction of amplitude. The latter effect is almost invariably obtained, with all strengths of stimulus above

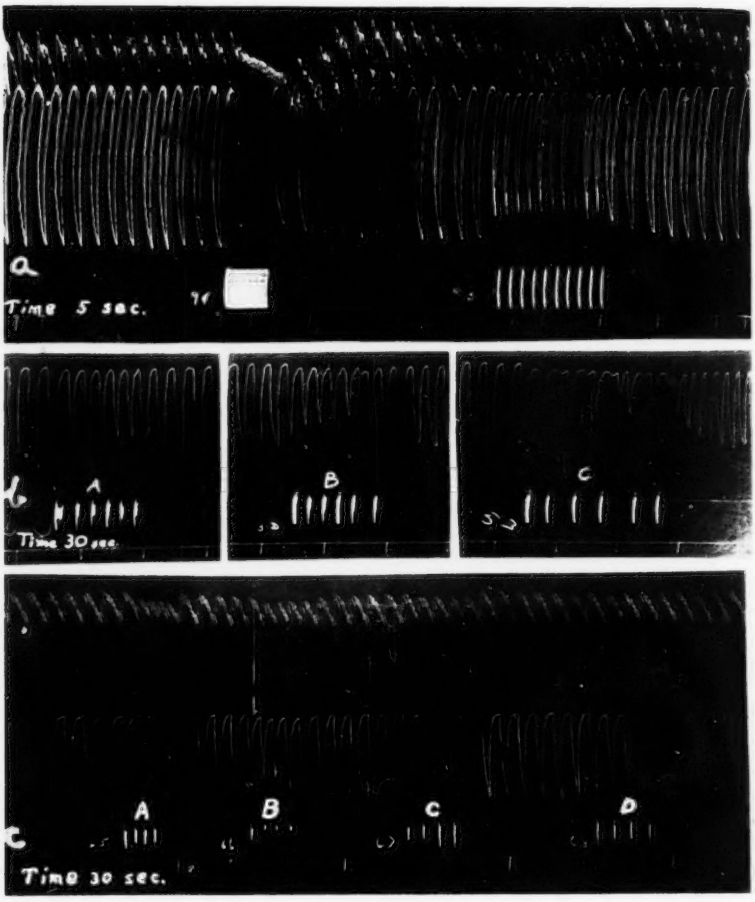

Fig. 1. a, male dog, 20.0 kilos, 6.0 grams barbital-sodium. Upper record, arterial pressure (carotid); lower, respiration. The down stroke, in this and all other records shown, is inspiratory. Showing continuous followed by intermittent stimulation of the central stump of the left vagus. Primary circuit 6 volts, secondary coil at 10 cm.

b, male dog, 9.0 kilos, 3.6 grams barbital-sodium. Intermittent stimulation of right vagus, mercury contact at constant level. Primary circuit, 6 volts. A, secondary coil at 10 cm., 45°; B, at 8 cm. horizontal; C, at 2 cm.

c, female dog, 19 kilos, 11.2 grams barbital-sodium. Intermittent stimulation of right vagus, secondary coil at 10 cm., 10°, mercury cup progressively lowered from A to D.

threshold. With four of seventeen dogs used, we occasionally saw the prolonged inspiration described by Gesell and Moyer (1935). In a series of ten or twelve cycles one or two inspirations (most often the first) would be of this type, the others abbreviated as usual. In one animal, the inspiratory response was abolished by giving more of the anesthetic. With the others this was not tried.

The acceleration was obtained with every dog, except one, of the series. It is most constant and uniform, however, when the intermittent stimuli used are relatively weak. With stronger stimuli the rhythm becomes irregular, and with further strengthening it is slowed (fig. 1b). In three cases an intermediate position of the secondary coil was found at which neither continuous nor intermittent stimulation of the vagus produced any visible effect upon the respiration. Tests with the oscillograph showed that the stimulating circuit was intact. Stronger and weaker stimuli had the usual effects.

No doubt all of the vagal afferent fibers were brought into activity by some of the stimuli employed. It might therefore be inferred that only the reduction in amplitude was due to the inhibitory fibers, the increase of frequency being caused by simultaneous stimulation of the augmentor group. This seems to us unlikely, for the following reasons. First, Hammouda and Wilson (1935a) conclude, from the resistance of the augmentor fibers to pressure block, that they are of relatively small size. If so, they should have a high threshold, and it ought to be possible, with sufficiently weak stimuli, to obtain the reduction in amplitude without the acceleration. Actually, as stated above, the weakest stimuli capable of inhibiting inspiration are most effective in accelerating. Second, the degree of acceleration varies with the position of the mercury contact, i.e., with the stage at which the inspirations are inhibited. The greater the reduction in amplitude, the greater the increase in frequency. Such a relationship seems to be implicit in Head's theory; it is difficult to explain if the two effects are attributed to different groups of afferent fibers (fig. 1c).

As shown in figure 1, the acceleration is usually immediate, following the first inhibited inspiration. For this reason, it can hardly be due to chemical stimuli affecting the center as a result of the reduced pulmonary ventilation. Acceleration from such a cause would be expected to develop gradually, with a certain delay, as does the slight rise of arterial pressure seen in figure 1a.

Tentatively, we suggest the following explanation; that inhibition, if just sufficiently intense to cut short inspiration, has a brief effect which does not continue far into the expiratory phase. The center is thus allowed to recover early following incomplete discharge, as Head believed. Stronger inhibitory stimuli have a more sustained action on the center, and delay the onset of the next inspiration. It is true that expiration

automatically breaks the contact, and that this occurs more promptly with strong than with weak stimuli (fig. 1b); but the period of tetanization is never reduced below a minimum set by the reflex time and by the inertia of the mechanical system.

Hammouda and Wilson (1935b) compared the respiratory effects of stimulating the vagus and superior laryngeal nerves. Both normally caused expiratory standstill. When the nerves were locally cooled at

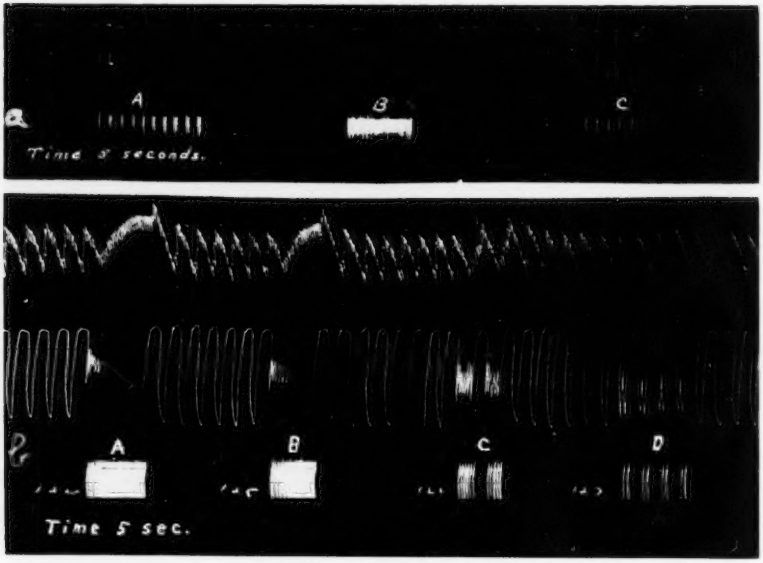

Fig. 2. a, Female dog, 7.7 kilos, 2.2 grams barbital-sodium, 30 mgm. morphine sulphate. Both vagi sectioned. Stimulation of right superior laryngeal, 6 volts in primary, secondary coil at 10 cm., 45°. B, continuous tetanization; A and C, intermittent stimulation, mercury cup lower at C.

b, same animal as in 1a. Intermittent stimulation of right superior laryngeal nerve. Primary circuit 6 volts, secondary coil at 8 cm. Mercury cup progressively lowered from A to D.

points central to the electrodes, the reflex from the superior laryngeal was simply lost at about 8°, with no effect at lower temperatures. The inhibitory effect of the vagus was also lost at the same temperature, but on further cooling an accelerator reflex was observed. The conclusion was drawn that "augmentation of the rate of breathing cannot under any conditions be due to the stimulation of fibers carrying inhibitory impulses."

We cannot see that the evidence offered warrants such a conclusion.

So far as the publication shows only continuous tetanization was employed, although the authors (1935a) apparently accept the common view that the normal activity of the vagal inhibitory fibers is periodic. Even if it had been proved that the superior laryngeal could not under any conditions accelerate breathing, it would not necessarily follow that the inhibitory fibers of the vagi are incapable of doing so.

It is true that the two nerves, continuously tetanized, produce similar effects, particularly if stimulation is begun during expiration. With intermittent stimulation, however, one striking difference appears. The vagus (fig. 1) cuts short inspiration abruptly, and expiration, even though it automatically cuts off the stimulus, is complete. Under the same conditions stimulation of the superior laryngeal causes the animal to expire just sufficiently to break the contact, and then inspiration is resumed. The lungs are not deflated to the original expiratory level until after one or more abortive attempts to complete the interrupted inspiration (fig. 2a). In spite of this, the frequency of "complete" cycles is augmented in this instance, and it has been in several others. More often, however, it is reduced, because each inspiration is drawn out into a series of small alternating inspiratory and expiratory movements. If these are counted as separate cycles, the frequency is greatly accelerated.

Figure 2b shows the results of intermittent stimulation applied to the superior laryngeal nerve at various stages of inspiration. In no case is the inspiration promptly terminated. It is more easily inhibited at an advanced stage than near the beginning, but there are always efforts to complete it. When the stimulus is applied early there may be six or seven such movements, ending in a period of tetanus. These effects are obtained with a wide range of stimulus strength, in animals with and without tracheotomy; and they are not materially changed by deepening the anesthesia almost to the point of respiratory failure.

This feature of the superior laryngeal reflex was quite unexpected, in view of the powerful expiratory effect ordinarily attributed to that nerve. It is possible to induce expiration if the stimulus is maintained. Clearly, however, the inhibitory fibers of the vagus and of the superior laryngeal do not affect the central mechanism of respiration in exactly the same manner.

SUMMARY

1. A stimulating circuit, controlled by changes in an animal's intrapleural pressure, is described. Contact is made at the desired stage of inspiration, and broken at the corresponding stage of expiration.

2. In dogs, anesthetized with barbital-sodium and with both vagi sectioned, stimulation of the central stump of one vagus in the manner described causes reduction of the amplitude of inspiration.

3. The rate of breathing is accelerated with the ordinary range of stimulus strength. Very strong shocks cause irregular slowing.

4. The degree of acceleration varies with the stage at which the inspirations are inhibited. The greater the reduction of amplitude, the greater the increase in frequency. These observations seem to indicate that inhibitory fibers may indirectly govern the frequency, in the manner suggested by Head.

5. Under these experimental conditions, stimulation of the vagus cuts short inspiration promptly and completely, while stimulation of the superior laryngeal does not. In the latter case, expiration does not occur until after one or more abortive efforts to complete the interrupted inspiration. In a number of instances, however, respiration has been accelerated through the superior laryngeal nerve.

REFERENCES

ADRIAN, E. D. J. Physiol. **79**: 332, 1933.
GESELL, R. AND C. MOYER. Quart. J. Exper. Physiol. **25**: 1, 1935.
HAMMOUDA, M. AND W. H. WILSON. J. Physiol. **74**: 81, 1932.
J. Physiol. **83**: 292, 1935.
J. Physiol. **85**: 62, 1935.
HEAD, H. J. Physiol. **10**: 279, 1889.

THE PHYSIOLOGICAL SIGNIFICANCE OF THE ELECTRIC RESPONSES OF SMOOTH MUSCLE

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In previous reports from this Laboratory (Rosenblueth, Leese and Lambert, 1933; Lambert and Rosenblueth, 1935) the electric responses of several smooth muscles to single nerve volleys have been described. In these communications tentative suggestions of possible functions of the different components of the electrograms have been submitted.

The preceding paper in this series (Rosenblueth and Cannon, 1936) describes the behavior of the smooth muscle electrograms after injection of several drugs, whose action on the mechanical responses is either known or therein studied. The present communication deals with the electrograms of the nictitating membrane and the pilomotors of the cat on repetitive stimulation of the preganglionic and the postganglionic nerve supply. Certain features of the mechanical responses of the nictitating membrane are described as a complement to the electric inquiry. Observations on the electrical excitability of denervated muscles are also included.

These data afford a basis for an attempt at interpreting the significance of the action potentials of smooth muscle.

METHOD. Cats were used, under dial anesthesia (Ciba, 0.7 to 0.8 cc. per kgm. intraperitoneally). The mechanical responses of the nictitating membrane (n.m.) were recorded isotonically or isometrically on a kymograph (cf. Rosenblueth, 1932).

For stimulation, shielded electrodes were applied to the nerves crushed or cut centrally. The electrical stimuli in some experiments were induction shocks from a balanced inductorium (Bishop, Erlanger and Gasser, 1926). The frequency of stimulation was varied by means of a rotary interrupter or by a mercury key operated by hand. In the experiments in which records were obtained by the cathode-ray oscillograph, stimulation was provided by a thyratron oscillator whose output was amplified and then delivered to the primary of a Harvard inductorium. The inductorium was found essential in order to reduce an otherwise excessive electrical artifact, apparently due to capacity between the thyratron circuit and the recording amplifier. The resulting shocks were diphasic, but they wholly subsided within the refractory period of the nerves. The frequency of the stimulating thyratron was controlled either by a second

thyratron circuit or by the rotary interrupter. The use of two independent circuits allowed completely independent variation of frequency and of intensity of the stimuli.

When stimulating the preganglionic sympathetic fibers to the n.m. the electrodes were applied to the cervical sympathetic nerve at about the level of the thyroid cartilage. The superior cervical ganglion was not dissected in these instances, to avoid a possible interference with its blood supply. For stimulation of the postganglionic fibers to the n.m. the branches of the superior cervical ganglion were dissected and the ganglion was crushed, to eliminate any possible preganglionic participation.

For stimulation of the pilomotors (p.m.) the electrodes were placed on the abdominal chains at about the level of L4. No special controls were made to find out whether the nerve supply to the hairs studied was pre- or postganglionic.

The electrodes used to lead the electric responses to the amplifiers were either silver-silver chloride agar-saline wicks or silver-silver chloride needles. The wick leads were employed exclusively as grid leads, the ground electrode being then a silver-silver chloride needle or plate inserted underneath the skin in a suitable "indifferent" region: the external canthus or the frontal region for the n.m., and the inguinal or pubic region for the p.m. The grid wicks were merely applied to the surface of the n.m. or the shaved skin of the tail.

When silver-silver chloride needles were used as grid leads, they were inserted as follows: into or through the n.m. near either of the inferior angles of the marginal cartilage; and intradermally or subcutaneously in the tail for the p.m.

Either a Hindle string galvanometer or a cathode-ray oscillograph was used for recording. For many of the experiments on the pilomotors, the string galvanometer, with no amplifier, proved adequately sensitive. In other experiments, the direct-coupled amplifier described by Garceau and Forbes (1934) was employed. In the most recent experiments another amplifier, constructed by Mr. A. M. Grass, was substituted. The cathode-ray oscillograph and associated amplifiers described by Garceau and Davis (1934) were used in some of the experiments. An input condenser of 1 μ F was connected in series with the grid of the first tube. This gave a half-time value of 1 second for the entire recording system.

RESULTS. *a. The electric responses of the n.m.* The results were qualitatively similar for both preganglionic and postganglionic stimulation of the nerve supply. The only quantitative difference worthy of mention was a tendency of the first component of the electrograms to sustain its magnitude better on repetitive postganglionic stimulation than on preganglionic stimulation. This is a minor difference and a single description will therefore serve for both cases.

In analyzing the electrograms of the n.m. on repetitive stimulation it is

important to recall that the electric response to a single shock consists of at least three components, I, II and III (Lambert and Rosenblueth, 1935). The first component, I, is a monophasic spike which lasts about 80 to 100 msec. The component II begins immediately after I and lasts about 350 msec.; it is frequently diphasic or polyphasic. Contraction begins during II. The component III is roughly coincident with the mechanical response.

In the present experiments I invariably denoted a positivity of the free border of the n.m. with respect to the body of the animal. The significance of this polarity will be discussed later. Our interest was primarily centered on the behavior of I and II.

Changes in the electrogram of the n.m. on repetitive stimulation appeared with frequencies as slow as 0.5 per second. At this rate of stimu-

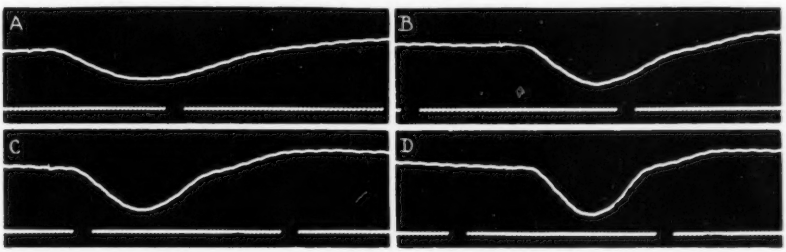

Fig. 1. Electric responses (I) of the nictitating membrane (n.m.). Cathode-ray oscillograph. Stimulation of the postganglionic fibers at a frequency of 0.5 per sec. The interruptions in the time signal denote 0.25 sec. intervals. Downward excursions of the spot denote positivity of the grid lead.

A to D, 1st to 4th responses.

lation, in the majority of the cases, there was a decline of I to about 60 per cent of the first response (fig. 2). In some cases, however, no change, or, rarely, even an increase of I, was apparent (fig. 1).

As regards the behavior of II at these slow frequencies, there was usually an increase of some of its phases with the successive stimuli, so that it was modified and became more prominent (fig. 2). In some preparations, when two stimuli were applied at a relatively long critical interval, II clearly began during I, completely modifying the electrogram.

From the long durations of I and II reported above, it is obvious that, when the frequency of stimulation is increased, the interval between the stimuli soon becomes shorter than the duration of these potentials. The succeeding I will then be superimposed on the previous II. In such cases I as a rule was decreased, while II increased or was not significantly altered.

The effects of higher frequencies may be generally described as follows. The component I invariably declined during a series, the faster the frequency the more rapidly. At frequencies of from 10 to 20 per second

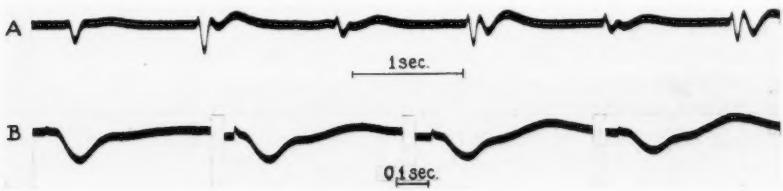

Fig. 2. Electric responses, I and II (string galvanometer), to series of stimuli (induction shocks) at a frequency of 1 per sec. In this and the succeeding string-galvanometer records downward excursions denote negativity of the ground lead; the moment of application of the stimulus is indicated by an arrow or by the artifact which precedes the responses.

A. Dial; 933F (2.5 mgm. per kgm.). Pilomotors (p.m.).

B. Dial. N.m. Responses to 1st to 4th break shocks applied to the preganglionic fibers.

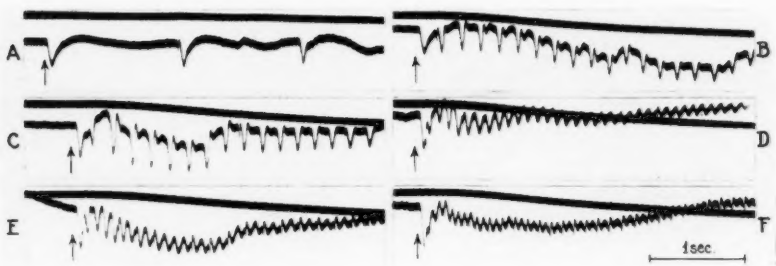

Fig. 3. Dial; cocaine (8 mgm. per kgm.); yohimbine (3 mgm. per kgm.). Electric responses of the n.m. (string galvanometer). Repetitive stimulation (induction shocks, rotary interrupter) of the postganglionic fibers.

A. Frequency, 0.8 per sec. Amplification, 1 mv. = 25 mm.
B. Frequency, 5 per sec. Amplification, 1 mv. = 25 mm.
C. Frequency, 5 per sec. Amplification, 1 mv. = 32 mm.
D. Frequency, 9 per sec. Amplification, 1 mv. = 32 mm.
E. Frequency, 9 per sec. Amplification, 1 mv. = 38 mm.
F. Frequency, 16 per sec. Amplification, 1 mv. = 38 mm.

The amplification is measured in millimeters on the original 35 mm. film.

usually only a few spikes were discernible at the beginning of the series. With higher frequencies no individual spikes were detectable. Independently of the frequency, the beginning of the electrogram reproduced in such cases the pattern of I and II as seen with a single shock—II, however,

showing summation. These initial changes were succeeded by sustained deflections, usually positive, to be followed by a prolonged negativity at the end of the series. The earlier portions of the sustained positive deflections clearly represent summated II waves merged smoothly together.

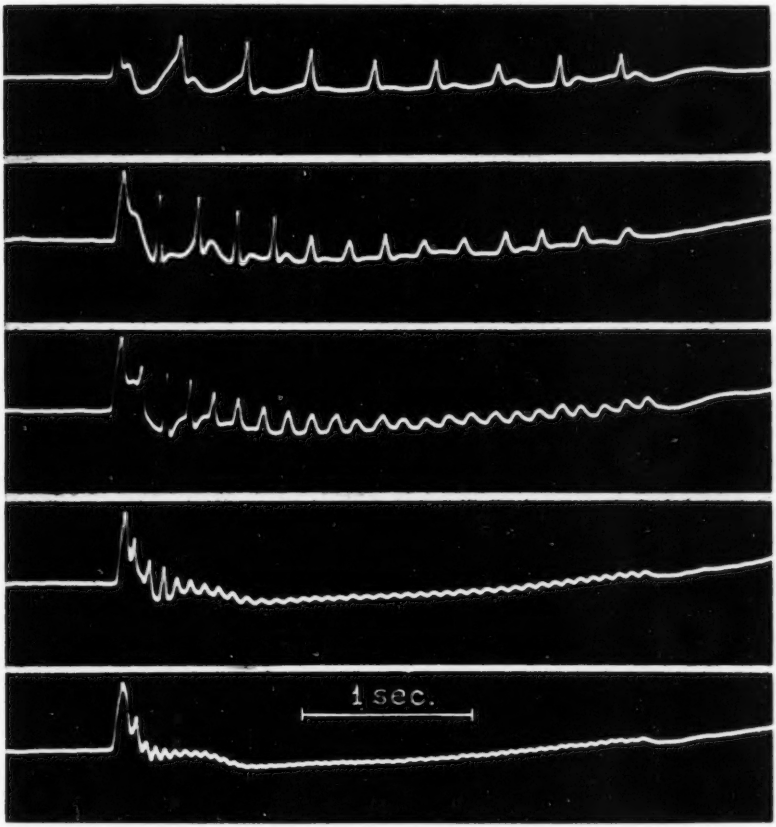

Fig. 4. Electric responses of the n.m. (cathode-ray oscillograph). Repetitive stimulation of the preganglionic nerve. Frequencies: 2.3, 4, 8, 11 and 16 per sec. Upward excursions of the spot denote positivity of the grid lead.

The transition from a treppe effect of II at very slow frequencies to a smooth summation at high frequencies may be followed continuously through the intermediate range. Component III naturally adds with the later portions of the sustained II responses. Figures 3 and 4 are typical.

Certain drugs, e.g., yohimbine (Rosenblueth and Cannon, 1936) or piperidinomethyl-3-benzodioxane (933F) (Monnier and Bacq, 1935; Rosenblueth and Cannon, 1936) have been shown to increase the action potentials, particularly I, of the n.m. and p.m. It was deemed of interest to study the effects of these drugs on the electric responses to repetitive stimulation. No significant difference was detected between the electrograms obtained before and after these substances at frequencies higher than about 4 per second (see Rosenblueth and Cannon, 1936, for the effects at low frequencies of stimulation).

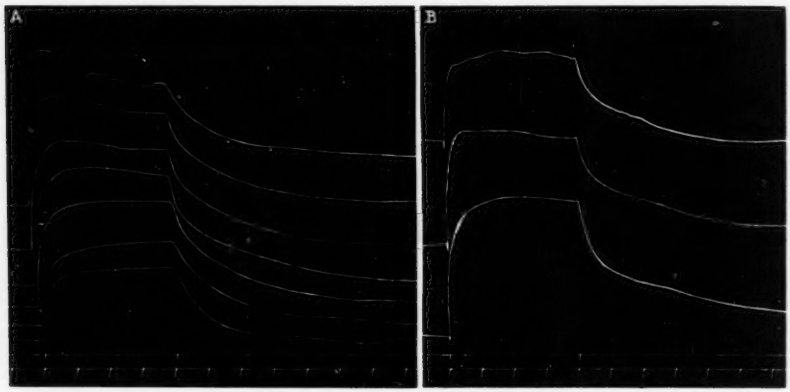

Fig. 5. Isotonic contractions of the n.m. The upper signal denotes the period of stimulation. The lower signal records 30 sec. intervals.

A. Preganglionic fibers stimulated. The records (from below upwards) are responses to the following frequencies: 4.5, 4.5, 10, 14, 19.5, 28.5 and 50 per sec. The order in which the observations were taken was the following: 4.5, 10, 14, 19.5, 28.5, 50, and 4.5 per sec.

B. Postganglienic fibers stimulated. Frequencies (from below upwards): 28.5, 50 and 100 per sec.

b. The mechanical responses of the n.m. Orías (1932) reported that when the preganglionic nerve supply to the n.m. is stimulated at relatively high frequencies (e.g., 40 per second) the isotonic record of the n.m. shows an initial rise to a maximum, succeeded by a slow decline although the stimulus be maintained. The responses to similar frequencies applied to the postganglionic fibers, on the other hand, are well sustained throughout stimulation.

Knoefel and Davis (1933) did not find any significant difference between pre- and postganglionic stimulation. The present results, however, confirm the report of Orías. Both isotonic and isometric records of postganglionic stimulation showed well-sustained responses up to frequencies

of about 100 per second. The records of preganglionic stimulation, on the other hand, showed unsustained responses with frequencies higher, as a rule, than 18 per second. As the frequency was further increased the maximum was reached earlier and the decline occurred sooner and to a lower level. Figures 5 and 6 illustrate typical instances.

Injections of 933F did not modify these results, except that the responses were always smaller after than before injection of the drug (fig. 6; cf. Bacq and Fredericq, 1935).

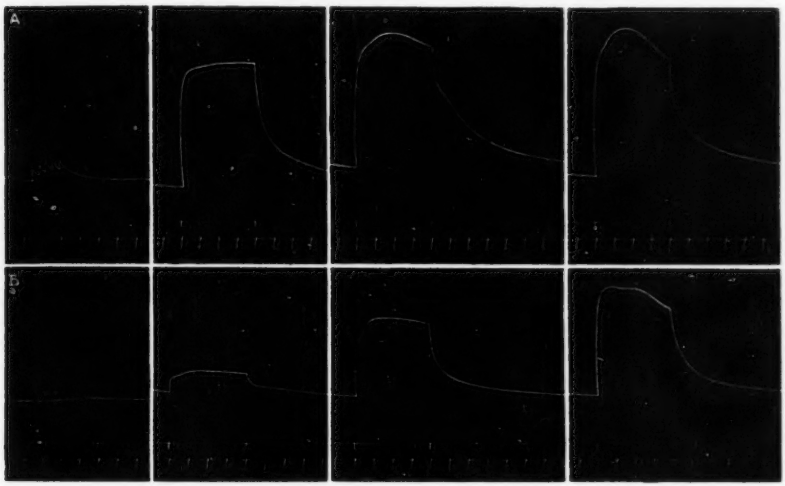

Fig. 6. Dial. Curare. Isotonic responses of the n.m. Time signal: 30 sec. intervals. Stimulation of the preganglionic fibers at the following frequencies: 0.1, 3, 11 and 18 per sec.

A. Before 933F.

B. After 933F (5 mgm. per kgm.).

c. The electrical excitability of the denervated n.m. Rosenblueth and Cannon (1934) reported that the denervated n.m. is electrically inexcitable. Monnier and Bacq (1935) confirmed this report but added that, in 4 cats, after injections of 933F, the denervated n.m. became electrically excitable.

We attempted in 3 cats to corroborate this appearance of electrical excitability after 933F. Our results were entirely negative. After doses of this drug of from 3 to 10 mgm. per kgm., as before the injections (Rosenblueth and Cannon, 1934), the denervated n.m. may contract slightly on strong repetitive stimulation. But these contractions differ entirely from the normal responses of the muscle to nerve impulses: they fail to show either temporal or spatial summation—i.e., variations in the intensity

(above threshold), duration, or frequency of the stimuli do not appreciably modify the contractions (fig. 7). We did not obtain any effects on single stimuli, even when direct current was applied for 5 seconds or more, and even when the e.m.f. was raised to 300 volts, so that visible damage to the tissues occurred. The muscles yielded normal graded responses to graded doses of adrenine even after these strong stimuli.

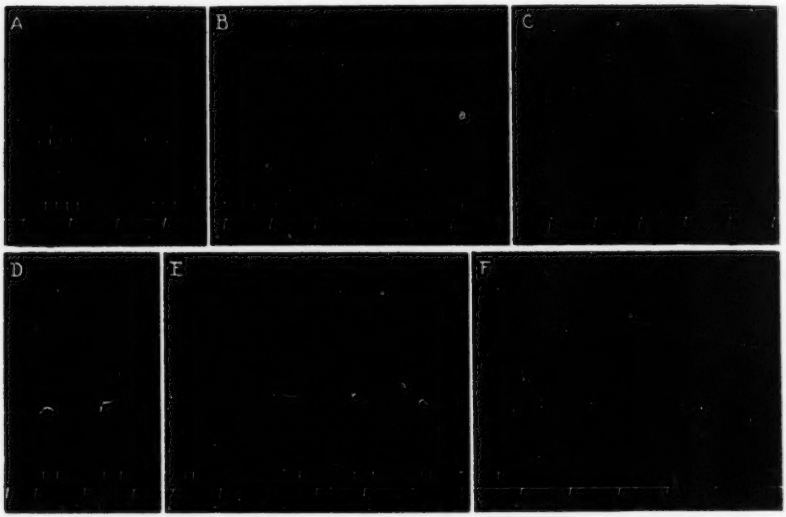

Fig. 7. Superior cervical sympathetic ganglion removed 12 days before. Direct stimulation of the n.m. Isotonic records. Time signal, 30 sec. intervals. The results of two experiments are illustrated; A, B, C and D, E, F, respectively.

A. Before 933F. Direct current pulses (0.2 sec.) applied as denoted by upper signals. Voltages: 20 and 40. The accidents in the record coincided with movements of the eye.

B. After 933F (2.5 mgm. per kgm.). Voltages: 20, 40 and 60.

C. Adrenalin (10 γ).

D. Dial, curare. Before 933F. Direct current pulses (0.02 sec.) applied as denoted by the upper signals. Frequency, 20 per sec. Voltage: 50.

E. Same as D, but after 933F (11 mgm. per kgm.). Voltages: 10, 20, 30, 50 and 50 (poles reversed).

F. Adrenalin (0.2 mgm.).

d. The electric responses of the pilomotors. With the position of the leading electrodes described under Method, that is, with the ground electrode placed on the abdominal wall, the sign of I in the p.m. was variable—i.e., the surface of the tail was sometimes positive, sometimes negative with respect to the abdomen. This variability was observed not only

from animal to animal, but, in two instances, the sign of I changed during the course of the experiment, although the leads had not been disturbed. Changing the position of the tail with respect to the animal did not alter the polarity of I. Likewise, changing either the ground or the grid needles to different positions in the abdominal walls or in the tail, respectively, failed to reverse the sign of the responses.

More consistent results were obtained when both leads were placed in the tail, as follows. The lead placed cephalad, whether grid or ground, usually became electropositive during I, with respect to the lead placed caudad. The other components, II and III, of the electrograms depended

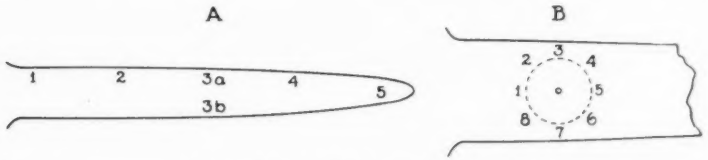

Fig. 8. Diagrams of points where the leads were inserted in the tail. For explanation see text.

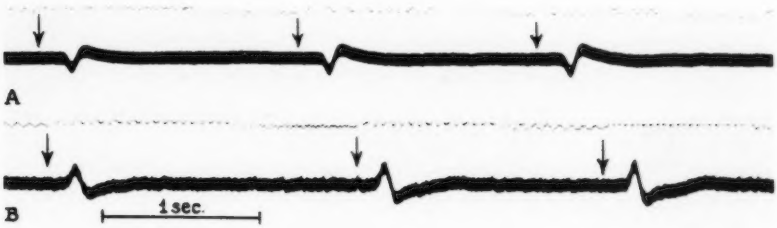

Fig. 9. Electric responses of the p.m. (string galvanometer). A square centimeter of skin was excised at the level of *2* in figure 8A and the grid lead was inserted into the underlying tissues.

A. Ground lead in position *3*.

B. Ground lead in position *1*.

also on the relative position of the leads along the longitudinal axis of the tail. Thus, if the grid needle was inserted at some point near the middle of the tail (position *3a* in fig. 8A), and the ground needle was placed first cephalad (positions *1* and *2*), then caudad (positions *4* and *5*), a complete reversal of all the phases of the electrogram occurred, so that mirror images were obtained (fig. 9). If both grid and ground were at the same level (*3a* and *3b*), no electric responses were detectable.

The magnitude of the electrograms varied with the distance (up to 10 or 15 cm.) between the leads. Thus, the grid being as before in position *3*, the e.m.f. of I when the ground was in *2* was smaller than that recorded

when the latter was in *1*; and similarly the responses increased when the ground was changed from *4* to *5*. If, however, one of the leads was placed at *1* or *5* and the other was successively implanted at progressively greater distances from the first, the responses, which at first increased, later decreased and became more complex in pattern (fig. 10). Invariably, when one of the leads was placed at the base of the tail (position *1*), the pattern of the responses was further complicated by the appearance of a swing preceding I and of opposite sign to this component (fig. 10).

The dependence of the magnitude and sign of the electrograms on the relative position of the electrodes with respect to the longitudinal axis of the tail was also strikingly shown as follows. Either the grid or the ground was placed in position *0* of figure 8B, while the other lead was placed successively at the points *1*, *2*, etc. In such cases *0–2* was found similar to but smaller than *0–1*; *0–3* gave no electrograms; *0–4* was a mirror image

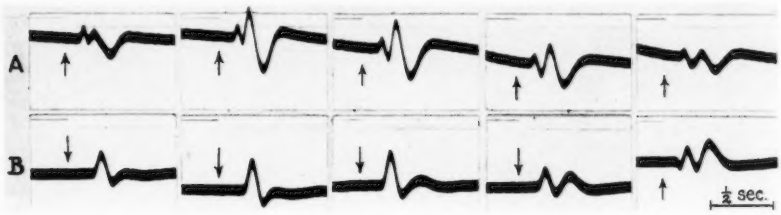

Fig. 10. Electric responses of the p.m. (string galvanometer).

A. Grid at base of tail. Ground successively inserted in positions *2*, *3*, *4*, etc., as in figure 8A. Distance between each two successive points of insertion, 5 cm.

B. Grid at tip of tail. Ground successively inserted in positions *5*, *4*, *3*, etc., as in figure 8A.

of *0–2*; *0–5* a mirror image of *0–1*; *0–6*, the same as *0–4*; *0–7*, no responses; and *0–8*, the same as *0–2*. When the positions *1–5*, *2–6*, *3–7*, etc., were tested, similar decreases and then reversed increases occurred. Figure 11 illustrates such tests.

In order to determine whether the tissue immediately in contact with the electrodes or the tissues included between them were responsible for the electrograms, we removed about a square centimeter of skin at one or both the points of contact. The records were practically identical whether both electrodes were in contact with uninjured skin, one on skin, another on bare muscle and connective tissue, or both on the underlying tissues (fig. 9). No modification comparable to the change from diphasic to monophasic response seen in skeletal muscle and nerve on damaging the tissue under one electrode could be observed.

If the pilomotor muscles between the leads were removed by excision

of the skin, the electric responses disappeared. It was found necessary, however, to excise all the skin of the corresponding region of the tail, for if only the ventral portion was excised, the leads being inserted into the ventral aspect of the tail, some electric responses persisted, although smaller than in the intact animals, showing that the pilomotors in the dorsal region contribute to the electrograms recorded.

If the distance between the leads was kept constant and the electrodes were displaced along the tail (e.g., *1–2; 2–3; 3–4; 4–5;* fig. 8A), the latency of the responses increased when the leads were moved caudad. The pattern also frequently underwent slight changes. Obviously the longer latencies were due to conduction in longer segments of the nerves. This method then affords a means of calculating the rate of conduction in these nerves. Such a calculation was made in 4 animals and yielded a rate of conduction of 1.5 to 4 m. per sec. These figures agree well with the rate

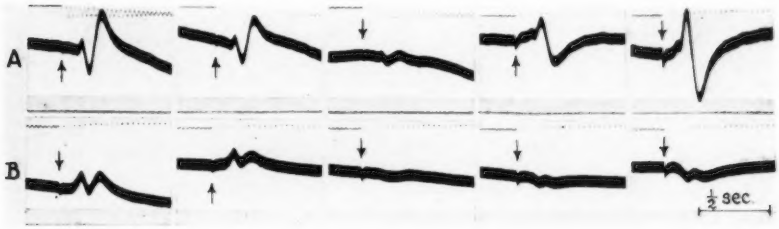

Fig. 11. Electric responses of the p.m. (string galvanometer).

A. Ground in position *0* as in figure 8B. Grid successively inserted in positions *1* to *5*. Radius: 1.2 cm.

B. Ground and grid rotated as follows: *1–5*; *2–6*; etc. (fig. 8B). Diameter 2 cm.

of conduction of the postganglionic pilomotor fibers in the cervical sympathetic, which was found by Eccles (1935) to be 1.7 to 5 m. per sec.

Slight, but definite electric responses were recorded when a ground plate was inserted underneath the skin and a grid needle was placed on the shaved surface, immediately above. With such leads the surface of the skin became negative with respect to the underlying tissues during I.

When two shocks were delivered at suitable time-intervals, the component II of the p.m. showed summation (cf. fig. 8, Lambert and Rosenblueth, 1935). Summation of II was not apparent in the n.m. with two shocks.

The component I of the electrogram of the p.m. is more stable on repetitive stimulation than in the n.m. Thus, in the n.m., a decline of I occurs at frequencies of stimulation slower than 0.5 per second (section *a*). In the p.m., on the other hand, it is usually necessary to reach frequencies of 1 to 2 per second before a decrease of I is visible.

Within the limitations imposed by these differences between the n.m. and the p.m., the electrograms of the latter on repetitive stimulation were similar to those obtained from the former. Figure 12 reproduces a typical instance.

c. The relations between the components I and II of the electrograms. A prominent characteristic of I is its tendency to decline on repetitive stimulation even at relatively slow frequencies—e.g., 0.5 per second for the n.m., 1 per second for the p.m. Its duration is practically impossible to determine, for II probably begins while I is developing and may modify the course of the latter. Thus, when the early phases of II increase during a series, I apparently shortens (fig. 2).

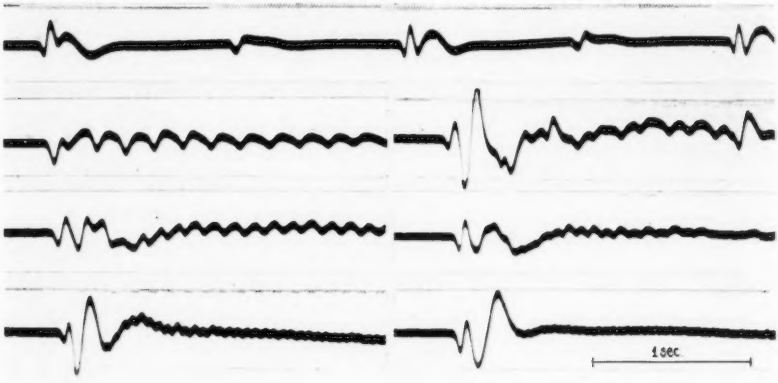

Fig. 12. Electric responses of the p.m. (string galvanometer). Repetive stimulation. Frequencies: 0.7, 5.5, 6.5, 8.3, 12, 17, and 22.5 per sec.

The component II is quite variable, even for single shocks separated by several seconds. It is in some preparations very prominent, but in other cases apparently absent, or masked by a large I. The ability of I to mask II was particularly striking in one experiment on the p.m. The effects of adrenine were being tested. This hormone decreases I, and, to a less extent, II, in both the n.m. and the p.m. (Rosenblueth and Cannon, 1936). In the case in question every time adrenine was injected I apparently reversed its sign, and then returned to its original direction. When the latencies of these responses were measured, however, it was found that the negative excursions before adrenine had a latency of 110 msec., while the positive deviations during the action of the hormone had a latency of 135 msec. Since in all other experiments with adrenine the latency of I was never changed, we interpret these results as due to the suppression of I, while the early onset of II became apparent.

DISCUSSION. A. *The method.* Our method for leading off the electric responses of smooth muscle has been criticized by Bacq and Monnier (1935). They used as electrodes a clamp made out of melted KCl, placed at the free border of the n.m. and a d'Arsonval-Lapicque wick placed on the surface of the n.m. at a point as distant from the other electrode as possible. They refer to the first one as an "inactive" electrode. They assume that the KCl depolarizes the underlying muscle, while the wick is in contact with active tissue.

Consideration of the anatomy of smooth muscle in general and the n.m. in particular will reveal the fallacy of these assumptions. According to Bacq and Monnier's description of their procedure, the KCl clamp was really in contact with the conjunctiva beneath which lay connective tissue and cartilage. The wick was also in contact with the conjunctiva, beneath which were connective tissue and some muscular elements. The majority of the muscle cells, however, were in reality between the leads, just as with the method we have adopted.

If this interpretation is correct, we should expect similar records with the two methods, and such is indeed the case. Although Monnier and Bacq (1935) describe the "action potentials" of the n.m. as monophasic, an examination of their published records (see figs. 2 and 3 of their paper) reveals that some of them are really polyphasic.

B. *The sign and magnitude of I.* From the data reported in section *d* (figs. 9, 10 and 11) it is obvious that the sign of I does not depend on whether the leads are in contact with muscular elements, but on the orientation of the active cells between the leads. This is what would be expected *a priori* when dealing with cells whose dimensions are smaller than the distance between the electrodes (cf. Adrian, 1925). For this reason we consider quite unnecessary the precaution which Monnier and Bacq (1935) stress, of attempting to depolarize a region of the muscle (e.g., by means of KCl). If local depolarization is to be effective it must depolarize a part of each individual muscle cell. Complete depolarization of some cells, while others are unaffected, is equivalent to changing the position of the physiological electrode.

Indeed, when the small size of the active elements is considered in relation to the distance between the electrodes, it is surprising that any electrogram is detected at all, for unless 1, the depolarization of each element is asymmetrical, either in time or degree, and also 2, unless the elements are similarly oriented, the electrodes would be isoelectric at all times. The first condition must be fulfilled if a muscle cell is to generate an external electrical field, and the second condition, if these fields are to reinforce instead of cancelling one another.

The important practical precaution is not the depolarization of the border of the n.m., but the lifting of the n.m. free from contact with the

eyeball. This greatly increases the recorded potentials by reducing the shunting effect of adjacent tissues.

We conclude that the smooth muscle electrogram is the resultant of an *oriented asymmetric* depolarization of the muscle cells. The data available are in accord with this view. Thus the component I is prominent in the n.m. and especially in the p.m., where the cells are known to be oriented, while it is comparatively small and difficult to detect in the bladder (Rosenblueth, Leese and Lambert, 1933; Lambert and Rosenblueth, 1935), where there is a conflicting orientation of the muscle cells. The fact that I in the n.m. was invariably found to denote positivity of the "active" grid lead does not necessarily mean that the polarization of the smooth muscle cells differs from that in nerve or skeletal muscle, but may be merely an expression of the orientation of the cells with respect to the leads. If the polarization in smooth muscle is similar to that of skeletal muscle, the direction of I implies that the depolarization begins in each cell nearer the base than the free border of the n.m.

The orientation and asymmetrical depolarization of the muscle cells is further substantiated by the increasing size of I in the p.m. when the number of effective elements is increased by augmenting the distance between the leads (fig. 10). The electric potentials of successive cells sum in series, much as those of the elements of the electric organs of fishes (cf. Gotch, 1900).

Since all three components of the p.m. electrogram were reversed when the relative position of the leads was reversed, and since this reversal was independent of whether or not either or both of the leads were in direct contact with "active tissues" (fig. 9), it follows that the sign of the responses does not justify any conclusions in relation to the sign of the changes in polarization of the individual muscle cells concerned. If a similar situation exists in the uterus, as is likely, it invalidates Bacq and Monnier's (1935) conclusion that the electric response (III) of the nonpregnant cat's uterus is a "Gaskell effect" denoting an increasing polarization of the muscle during the relaxation induced by hypograstric stimulation or by adrenine.

Orientation of the muscle cells in the n.m. and the p.m. is established anatomically. On the other hand, no anatomical data are available that may be correlated with the asymmetrical depolarization of each cell that we have inferred. It is possible that the region of entrance and intracellular termination of the nerves (see Cannon, 1933, for references) might furnish a clue for this asymmetry, but the question must be settled by future anatomical investigation.

The pilomotor system is more complex than the nictitating membrane. In the latter, a group of similarly oriented cells between the electrodes are activated almost simultaneously. In the former, many groups of similarly

oriented cells are distributed throughout the skin, both between and beyond the electrodes. They are not all activated simultaneously. A wave of pilomotor activity advances down the tail at a rate of 1.5 to 4 m. per sec.

The probable orientation of the pilomotor muscle cells is indicated in figure 13. We find (p. 398) that the skin surface becomes electrically negative during I. This implies that the end of each group which is superficial *and also caudal* becomes relatively negative and the deep, cephalad

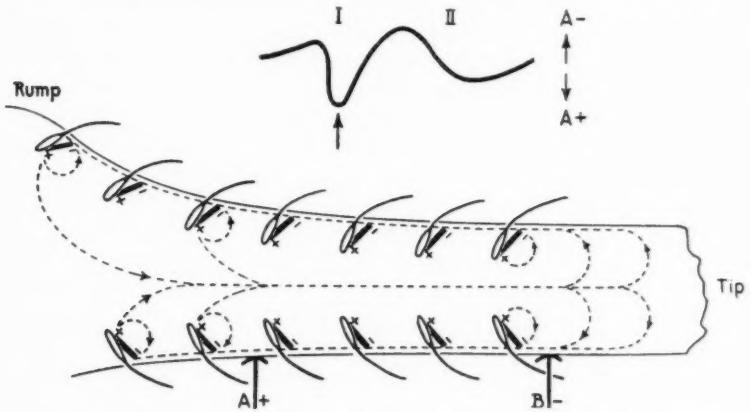

Fig. 13. Schematic representation of the p.m. of the tail. The orientation of the pilomotor muscles is shown by the oblique heavy bands. On activation, the superficial caudad end of each muscle becomes negative relative to the deep cephalad end. The diagram represents the moment when the muscles as far as B have become active. The corresponding time in the electrogram above is shown by the arrow. The active muscles constitute a series of electrical doublets, and the resulting direction of current flow and potential gradient is indicated by the dotted lines. Electrode A is positive relative to B, due to the summated effect of the longitudinal components of the doublets between them. At this moment, before units beyond B are activated, B would be negative relative to another more distal electrode (not drawn), due to the potential gradient indicated by the dotted lines in advance of the active muscles. This is the basis of the "leading negative phase" described in the text.

end, positive. This is what we usually observe (p. 396) in the simplest situation.

The leading negative phase (section *d*), best seen when the cephalad electrode is near the base of the tail, can be interpreted as analogous to the leading positive phase seen with skeletal or cardiac muscle in a volume conductor (Craib, 1928; Bishop and Gilson, 1929; Wilson, McLeod and Barker, 1933) As the wave of activity approaches over the skin of the rump and the base of the tail, it consists of a series of electrical doublets with negative pole directed toward the caudally located pair of electrodes

(fig. 13). The cephalad electrode A becomes relatively negative. A tapering mass of tissue between the electrodes will favor the appearance of this potential difference between A and B. When activity spreads past A, the potential difference between A and B abruptly decreases. As additional elements between A and B are activated they tend to make A relatively positive, soon overcoming the negativity produced by the elements central to A. A becomes more and more positive until the wave front reaches B. From then on, the subsequent phenomena are unpredictable unless we know the time course of the electrical events in a typical element. In so far as the elements are acting simultaneously, all those between A and B tend to sum their electrical effects like the cells of a battery, and are opposed by (usually much weaker) components, opposite in sign, contributed by the extra-polar regions cephalad to A and caudad to B. These will usually be recognized only when activity between A and B is slight or *nil*, as during the preliminary negative phase.

If the electrical sign of II is opposite to I and begins early enough (p. 399), it may modify the description given above, when the distance between A and B is great, by terminating the major positive swing before the wave of activity reaches B. If it were not for this limitation the time between the first negative and the first positive peaks in the electrogram would furnish an immediate basis for calculation of the velocity of conduction between A and B. As it is, we must base these calculations on differences in the latency of the *beginning of the first positive swing* of the cephalad electrode, irrespective of whether the leading negative phase is visible or not. This event denotes the onset of electrical activity under electrode A.

The apparently variable sign of I (p. 395) is thus partly explained. The size of the leading negative phase will vary with the degree to which the hairs on the rump are activated in a given case, and with any anatomical factors which might modify the distribution of potential in the tissues of the animal.

When the ground lead was in contact with the pelvic or inguinal regions the sign of I was sometimes positive, sometimes negative, and the leading negative phase was absent. In this case the relation of the active tissue to the leads is more complicated. The wave of pilomotor activity begins *between* the leads. The situation is too complex to render profitable any present attempts at further theoretical analysis. Likewise we refrain from describing in detail, much less interpreting, the occasional large diphasic or polyphasic responses seen during repetitive stimulation in a few experiments. They are puzzling exceptions to an otherwise regular series of typical responses.

C. *The significance of the electric responses in smooth muscle.* Purely on the basis of analogy we have previously assumed that I was the strict

equivalent of the spike potential of skeletal muscle (Rosenblueth, Leese and Lambert, 1933; Rosenblueth, 1935; Lambert and Rosenblueth, 1935). It is probable that I, like the spike of skeletal muscle, is due to a depolarization of some polarized interface. The electrical analogy would therefore be legitimate. The spike potential of skeletal muscle, however, denotes a conducted wave of depolarization; indeed, conduction is probably its chief function. In smooth muscles, on the other hand, electrical conduction of an excitatory state is improbable. The cells are small, the contractile process is slow; diffusion of the chemical mediator can therefore readily account for the phenomena observed. That there is no all-or-none intercellular conduction in smooth muscle was shown by Rosenblueth and Rioch (1933). The electrical inexcitability of denervated smooth muscles (section *c*) speaks likewise against the existence of an all-or-none wave of depolarization in these muscles.

The analogy between I and the spike potential of skeletal muscle breaks down in one significant respect. At frequencies of stimulation far below the optimal (20 to 25 per second) for contraction of smooth muscles, the I component shrinks to undetectable levels within a second or two. In skeletal muscle, even at frequencies so high as to cause rapid fatigue, the spikes remain clearly recognizable (Davis and Davis, 1932). Incidentally, the failure of this analogy renders meaningless the interpretation of "pseudo-fatigue" of smooth muscle offered by Knoefel and Davis (1933).

If I does not represent conduction, what may be its meaning? The depressing action of adrenine on this component (Rosenblueth and Cannon, 1936) furnishes a clue. This decline of the electrogram during the action of the hormone would indicate that adrenine partly or fully depolarizes the interface responsible for I. If the adrenine-like mediator is liberated inside some cells on stimulation of the sympathetic nerves, as is probably the case (see Cannon, 1933), then, this mediator or its compound (sympathin) with the receptive substance may also depolarize the interface, thus giving rise to I.

The latencies of I in both the n.m. and the p.m. are strongly in favor of this view. These latencies (Rosenblueth and Cannon, 1936) were: n.m. (postganglionic stimulation), 28 msec.; p.m., 120 msec. The distances between the stimulating electrodes and the muscles were about 6 and 12 cm., respectively. The rate of conduction of the postganglionic motor fibers of the n.m. is about 6 m. per sec. (Eccles, 1935). For the p.m. the rate of nerve conduction is about 1.5 to 4 m. per sec. (section *d*; the ganglionic delay, if preganglionic fibers were stimulated, is negligible for this calculation). We then find a delay of 18 msec. after the nerve impulse has reached the muscle, before I appears in the n.m., and a similar delay of at least 40 msec. in the p.m. Such large values cannot be accounted for

reasonably by the assumption of a slowed conduction at the fine nerve terminals. They may be readily explained, however, by the hypothesis suggested, as due to the time necessary for the liberation and diffusion of the mediator before the action potential begins.

The rapid decline of I on repetitive stimulation, even at relatively slow frequencies (sections *a* and *d*) is very difficult to reconcile with conduction on the analogy with skeletal muscle, but is readily explained by the present hypothesis. For, the mediator being destroyed only slowly, successive impulses, and the corresponding quanta of mediator, would encounter an increasingly depolarized interface.

Whether I is an all-or-none reaction is an open question, since no direct evidence is available. If the interpretation suggested above is valid then I need not follow the all-or-none principle, for we have assumed I to denote a depolarization of some interface, and have further assumed that the mediator is responsible for this depolarization and that the latter is proportional to the amount of mediator present.

The component II, being more variable and complex than I, is more difficult to interpret. Indeed, there is no reason to assume that it is a single phenomenon; its behavior (sections *a*, *d* and *e*) rather suggests that it may be due to a succession of several overlapping events. In nerve, the after-potentials have similarly been assumed by Gasser (1935) to be due to a chain of chemical reactions.

Our data show that II is clearly distinct from I. At slow frequencies of stimulation I may shrink while II increases (fig. 2). Adrenine sometimes has a similar differential effect (section *e*). At moderately slow frequencies II appears progressively earlier during I, often curtailing and reducing the latter. This may be another aspect of the differential growth of II, or else it implies a reduced latency. Since the latency of I is constant, this alternative also implies independence. Finally, at higher frequencies after the initial response, I characteristically shrinks to extinction, while successive II waves show a smooth summation.

The interpretation adopted previously for I has the following consequences as regards II. Since only some of the smooth muscle cells are innervated (see Cannon, 1933, for references), after the depolarization of the key-cells by the mediator, the latter would reach, in diffusing outwards, some of the non-innervated elements, and depolarize them in turn. This process might account for at least some of the phases of II. There probably are other factors at play, however, for 933F, which renders the smooth muscle less permeable leads frequently to an increase of II (Rosenblueth and Cannon, 1936). Decreased permeability might, however, be offset by greater polarization.

As regards III, we have nothing to add here to the discussion offered by Lambert and Rosenblueth (1935).

These hypotheses concerning the functions of the potentials in the n.m. and the p.m. may be summarized in the following schema (modified from Rosenblueth, 1935):

(1) Nerve action potential
↓
(2) Liberation of an adrenine-like mediator inside the innervated cells.
↓
(3) Combination of the mediator with the receptive substance.
↓
I ← (4) Depolarization of the surface of the key cells, and
(5) diffusion of the mediator and the compound (sympathin E).
↓
II ← (6) Depolarization of adjacent non-innervated cells, and
(7) penetration of the mediator;
↓
II (?) ← (8) further combination with the receptive substance in these cells.
↓
III ← (9) Depolarization of other interfaces (see Dubuisson, 1935) and contraction.

D. *The electrical excitability of the n.m.* In skeletal muscle the general interpretation is that the electrical stimulus sets up a local depolarization (local excitatory state); if this is sufficient a wave of depolarization, which is conducted (conducted disturbance) is initiated; this, in turn, elicits contraction by a mechanism which is still obscure.

The rôle we have attributed to I in smooth muscle excludes the conducted disturbance, an indispensable step in electrical excitation in terms of the generally accepted membrane theory. The fact that denervated smooth muscle is inexcitable (see section *c*) is in agreement with this interpretation. We are thus led to the inference that for an electrical stimulus to be effective in smooth muscle it must elicit the liberation of the chemical mediator (see also the Discussion, Rosenblueth and Cannon, 1936).

The electrical excitability of the denervated n.m. after 933F, reported by Monnier and Bacq (1935), which we have failed to confirm (section *c*), may merely imply that Monnier and Bacq attach to the term "electrical excitability" a meaning different from that which we have adopted here and which we believe is the standard meaning. They do not mention whether, in their 4 cats, they used single or repetitive stimulation to determine the strength-duration curves they publish. They do not state whether threshold, or submaximal, or maximal responses were used as an end-point. They do not comment on the characteristics of the responses, nor do they give any records from which these characteristics might be judged. It is possible that the responses they obtained were the small contractures we observed (fig. 7), or else, since they worked on uncurarized animals, that eye movements may have occasioned an artifactual movement of the n.m.

If, for these reasons, we dismiss the data of Monnier and Bacq as inconclusive in this respect, the only evidence available, to our knowledge, that might denote a direct electrical excitability of smooth muscle is the evidence presented by Simeone and Rosenblueth (1934), that the strength-duration curves of innervated n.ms. demonstrate two different excitabilities. Simeone and Rosenblueth attributed one of these excitabilities to nerve, the other to the muscle. It is possible, however, that the second excitability is that of the mediator-secreting structure, which would depend trophically on the nerve, and therefore degenerate after denervation. Whether smooth muscle is at all directly excitable electrically is therefore still an open question.

E. *The sympathetic ganglion.* Our results (sections *a* and *b*) are in full agreement with the now generally accepted notion that the ganglion acts primarily as a spatial (not temporal) multiplier, at least at low frequencies of stimulation of the preganglionic fibers.

The slight tendency of I to sustain its magnitude better on repetitive postganglionic than on preganglionic stimulation (section *a*) might be explained as due to the temporal dispersion imposed by the ganglion on the volleys which traverse it (Bronk, Tower and Solandt, 1935; Rosenblueth and Cannon, 1936).

The differences in the mechanical responses to high frequencies of stimulation of the preganglionic and postganglionic nerves, respectively (section *b*), reveal properties of the ganglion which require further study for interpretation.

SUMMARY

Mechanical and electric responses of the nictitating membrane and pilomotors of the cat were recorded on repetitive stimulation at various frequencies of the pre- and postganglionic nerve supply. The electrical excitability of the denervated nictitating membrane was studied. The effects of certain drugs on these phenomena were observed. The following conclusions are derived.

1. The electrograms denote an asymmetric depolarization of oriented smooth muscle cells (p. 401).

2. The polarity of the electrograms depends on the orientation of the muscles with respect to the electrodes (p. 401).

3. Therefore, the polarity of the electrograms of groups of cells does not give any information as to the sign of the electric changes in the individual cells (p. 401).

4. The electric potentials of neighboring cells may add in series (p. 403).

5. The spike component (I) of the electrograms is probably produced by the chemical mediator (p. 404).

6. Therefore I is not a propagated disturbance (p. 404).

7. It may not follow the all-or-none law (p. 405).

8. Smooth muscle may be electrically inexcitable (p. 407).

9. The conclusions 5, 6, 7 and 8 reveal fundamental differences between smooth and skeletal muscle.

The probable sequence of events in neuromuscular conduction in smooth muscles is summarized (p. 406).

REFERENCES

ADRIAN, E. D. J. Physiol. **60:** 301, 1925.

BACQ, Z. M. AND H. FREDERICQ. Arch. Internat. Physiol. **40:** 454, 1935.

BACQ, Z. M. AND A. M. MONNIER. Ibid. **40:** 467, 1935.

BISHOP, G. H., J. ERLANGER AND H. S. GASSER. Ibid. **78:** 592, 1926.

BISHOP, G. H. AND A. S. GILSON. This Journal **89:** 135, 1929.

BRONK, D. W., S. S. TOWER AND D. Y. SOLANDT. Proc. Soc. Exper. Biol. Med. **32:** 1659, 1935.

CANNON, W. B. Science **78:** 43, 1933.

CRAIB, W. H. J. Physiol. **66:** 49, 1928.

DAVIS, H. AND P. A. DAVIS. This Journal **101:** 339, 1932.

DUBUISSON, M. Arch. Internat. Physiol. **41:** 511, 1935.

ECCLES, J. C. J. Physiol. **85:** 179, 1935.

GARCEAU, L. E. AND H. DAVIS. This Journal **107:** 305, 1934.

GARCEAU, L. E. AND A. FORBES. Rev. Sci. Instruments **5:** 10, 1934.

GASSER, H. S. This Journal **111:** 35, 1935.

GOTCH, F. Schäfer's Text book of physiology, II, 561. Edinburgh, 1900.

KNOEFEL, P. AND H. DAVIS. This Journal **104:** 81, 1933.

LAMBERT, E. AND A. ROSENBLUETH. Ibid. **114:** 147, 1935.

MONNIER, A. M. AND Z. M. BACQ. Arch. Internat. Physiol. **40:** 485, 1935.

ORÍAS, O. This Journal **102:** 87, 1932.

ROSENBLUETH, A. Ibid. **102:** 12, 1932.
Quart. Rev. Biol. **10:** 334, 1935.

ROSENBLUETH, A. AND W. B. CANNON. This Journal **108:** 384, 1934.
Ibid. **116:** 415, 1936.

ROSENBLUETH, A., C. LEESE AND E. LAMBERT. Ibid. **103:** 659, 1933.

ROSENBLUETH, A. AND D. MCK. RIOCH. Ibid. **106:** 365, 1933.

SIMEONE, F. A. AND A. ROSENBLUETH. This Journal **110:** 399, 1934.

WILSON, F. N., A. G. MCLEOD AND P. S. BARKER. The distribution of the currents of action and of injury displayed by heart muscle and other excitable tissues. Ann Arbor, 1933.

THE SENSITIZATION OF A SYMPATHETIC GANGLION BY PREGANGLIONIC DENERVATION

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Meltzer and Auer (1904) noted that adrenalin causes a greater contraction of the blood vessels of the rabbit's ear, the nictitating membrane and the dilator fibers of the iris when these structures are denervated than when they are normally innervated. Elliott (1905) confirmed and extended these observations, including evidence that the retractor penis and the pilomotors, when denervated, are made specially responsive to injected adrenalin. Other observers (Lichtwitz and Hirsch, 1910; Pearce, 1913; and Shimidzu, 1924) have reported similar phenomena. Shimidzu published records showing a greater efficacy of adrenalin in producing inhibition of denervated than of innervated rabbit intestine. Meltzer and Auer and also Elliott noted that the effect was not immediate but developed some days after the denervation. The course of the increasing response of the nictitating membrane after removal of the superior cervical ganglion was recorded by Hampel (1935), who found that the contractions resulting from standard doses of adrenalin rose rapidly during the first six to eight days, more slowly during the six to eight days which followed, and that after a maximal responsiveness was reached no further change occurred. He drew the same conclusion that Elliott had drawn, that the increase of the response was due to an increase of sensitivity to injected adrenalin.

Sympathetic impulses are mediated by an adrenine-like substance (sympathin) and parasympathetic impulses are mediated by acetylcholine. Since sympathetic denervation increases the effects of adrenine, the question arose as to whether degeneration of the parasympathetic supply to a smooth muscle would sensitize this muscle to acetylcholine. An affirmative answer to this question was provided by the observations of Shen and Cannon (1936), who found that after removal of the ciliary ganglion the constrictor muscle of the iris became highly sensitized to acetylcholine.

Dale and Feldberg (1934) have found indications that in the transfer of impulses from motor nerves to skeletal muscle there is an intervention of acetylcholine. When skeletal muscle is denervated it becomes sensitized to acetylcholine. Observations on both smooth and skeletal muscles,

therefore, suggest that denervation augments their response to the natural chemical mediators of the nerve impulses which affect them. It may be a general law that denervation increases sensitiveness to stimulating agents. The interesting question arose as to whether this would prove true also of nerve cells.

The outlying neurones with cell bodies in sympathetic ganglia may be regarded as being innervated by preganglionic fibers. Evidence has accumulated that in the transmission of nerve impulses from preganglionic fibers to ganglion cells acetylcholine serves as a mediator (Kibjakov, 1933; Feldberg and Gaddum, 1934). Specifically the question may be asked, does denervation of the ganglion cells by severance of the preganglionic fibers render them more sensitive to acetylcholine? An answer to this question was the object of the present investigation.

METHOD. The nictitating membrane of the cat was employed as an indicator. The preganglionic fibers in the cervical sympathetic supply were aseptically severed on the right side, and at least a week was allowed for degeneration. At the time of the experiments dial (0.8 cc. per kgm.) was used as an anesthetic. The preganglionic fibers on the left side were cut acutely, and both nictitating membranes were arranged for recording. Two methods of testing increased sensitiveness of the ganglion to acetylcholine were employed, injection and local application.

For the first method the adrenal glands were removed in order to avoid possible action of adrenine secreted because of injected acetylcholine (Feldberg, Minz and Tsudzimura, 1934). Atropine (1 mgm. per kgm.) was administered intravenously to lessen the effects of acetylcholine on the membranes (Rosenblueth, 1932) and on the blood pressure; it has no significant influence on the ganglion cells. A small dose of curare was used to stop the eye movements caused by acetylcholine (Duke-Elder and Duke-Elder, 1931) and also to obviate the muscular quivering from injected prostigmin; it lessens somewhat the sensitiveness of the ganglion, but not to a great extent (Langley and Anderson, 1895). Finally, prostigmin (Roche; 0.5 mgm. per kgm.) was injected to protect acetylcholine from destruction by esterase in the circulating blood.

In the later experiments the second method was employed, in which no preparatory drugs were injected, and small pledgets of cotton wet with acetylcholine in concentrations varying from 1 to 10 per cent were applied directly to the superior cervical ganglia laid bare *in situ*.

RESULTS. In figure 1 are presented the tracings of the nictitating membranes recording simultaneously, the right above, the left below, in each instance. The first part of figure 1A confirms the observation made by both Elliott and Hampel, that severance of preganglionic fibers will in time sensitize the paralyzed muscle; the right (chronically decentralized) membrane contracts more than the left in response to 0.005 mgm. adrena-

lin. The second part of figure 1A presents two responses of the membranes to acetylcholine, 0.3 and 0.5 mgm. respectively. The right membrane after each injection contracted sharply; in the left membrane there was no response; the slight change was possibly due to a movement of the eyeball.

Figure 1B shows the responses which occurred when the same procedures were used after both superior cervical ganglia had been removed. Adrenalin had the same effect as before, as was expected. When the doses of

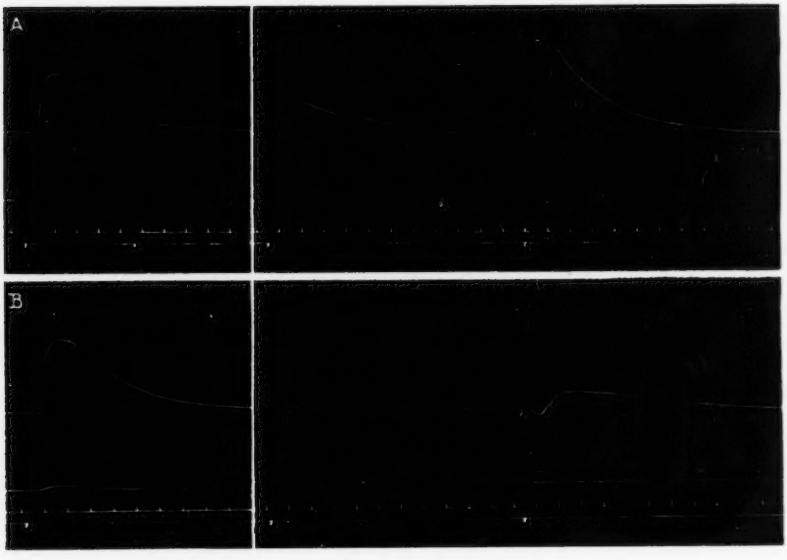

Fig. 1. Cat. Dial, atropine, curare prostigmin. Adrenals ligated. Right superior cervical sympathetic ganglion denervated 9 days. Records of nictitating membranes: right, above; left, below. Effects of injecting (1) adrenalin, 0.005 mgm.; (2) acetylcholine, 0.3 mgm.; and (3) 0.5 mgm. A, before, B, after removal of superior cervical ganglia.

acetylcholine were repeated, however, the quick contraction of the membrane on the denervated side entirely disappeared and the heights of the contractions were much less as compared with the heights when the ganglia were present. From these and other similar observations the inference is justified that the quick and high response of the membrane supplied by the denervated ganglion cells was due to a discharge from these cells; and since it did not occur on the side where the ganglion cells were acutely denervated, the cells on the chronically denervated side must have

acquired with the passage of time an increased sensitiveness to acetylcholine. As has been shown by Rosenblueth (1932), acetylcholine can by itself cause a contraction of the nictitating membrane. The records in figure 1B prove that the membrane becomes sensitized to acetylcholine after severance of the preganglionic fibers which normally bring impulses to it.

Figure 2A presents the results typically obtained when acetylcholine was applied directly to the right, chronically denervated superior cervical ganglion (upper record) and to the left, acutely denervated ganglion (lower record). There was a striking response on the right side and a negligible response on the left. That the nictitating membrane on the left was quite capable of contraction is shown in figure 2B, a record of the effect of a small dose of adrenalin injected intravenously.

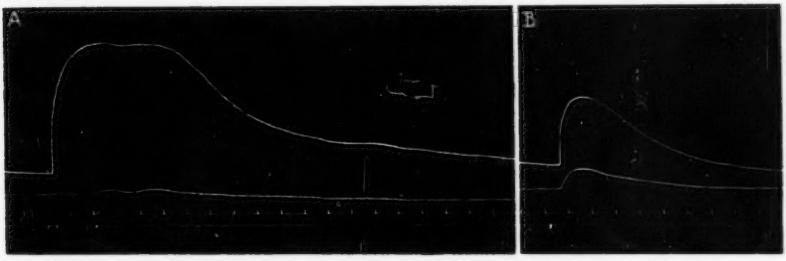

Fig. 2. Cat. Dial. Right superior cervical sympathetic ganglion denervated 15 days. Records of the nictitating membranes: right, above; left, below. A, acetylcholine (1 per cent on cotton pledgets) applied to superior cervical ganglia: first pair of marks, pledgets on; second pair, off. B, adrenalin, 0.005 mgm. intravenously.

The possibility that there might be a transport of acetylcholine in the blood stream from the region of the ganglion to the nictitating membrane was tested by removing both superior cervical ganglia and placing pledgets wet with acetylcholine in the same places as before. The results were negative.

General considerations. The question arises as to how the increased sensitiveness of denervated structures may be explained. It might be supposed that innervated cells require a continuous minimal supply of the chemical agent which directly induces their response in order that they may be normal. If this chemical agent should be lacking, the cells might be influenced by a smaller amount than would be needed naturally. The efficacy of small doses of thyroxin in myxedema and insulin in diabetes, as compared with the large doses necessary to produce changes in the normal individual, might be cited in support of such a view. Obviously, however, this view would require the denervated structures to

be specifically sensitive to the agent which is lacking, i.e., to the agent which is normally active.

An alternative suggestion is that the greater sensitiveness is due to an increased permeability of cell membranes when they are denervated. If this is true, then a given chemical agent effective as a stimulus would enter faster because of a readier access to the interior of the cell, i.e., the concentration inside a denervated cell would be greater than in normal cells in the same circumstances. Furthermore, if the suggestion of greater permeability is true, it should be true not only for natural stimulating agents, but also for other agents which can be applied. This has proved to be the case. Skeletal muscle, deprived of its nerve supply, becomes specially responsive to the action of not only acetylcholine, but also potassium chloride, nicotine and other substances belonging to the nicotine group (see Dale and Gasser, 1926). In smooth muscle, likewise,

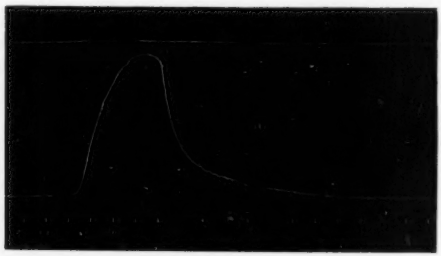

Fig. 3. Cat. Dial. Right superior cervical sympathetic ganglion denervated 32 days. Records of nictitating membranes: left, above; right, below. Potassium chloride (5 per cent on cotton pledgets) applied to superior cervical ganglia: first pair of marks, pledgets on; second pair, off.

acetylcholine, pilocarpine and physostigmine, in addition to adrenine, all cause a greater contraction of the nictitating membrane when chronically denervated than when freshly denervated (Rosenblueth, 1932). Pilocarpine as well as acetylcholine is extra-active on the iris constrictors deprived of their nerve supply (Anderson, 1905). This non-specificity is seen also in denervated ganglion cells. As Brown and Feldberg (1935) have pointed out, potassium ions stimulate ganglion cells. In figure 3 are presented the records of the nictitating membranes when 5 per cent potassium chloride was applied to the chronically denervated right superior cervical ganglion (lower record), and simultaneously, to the left acutely denervated ganglion (upper record). Obviously the potassium ions had a much greater effect on the chronically denervated cells. The evidence at hand supports the theory that severance of their nerves renders the denervated structures more permeable to stimulating agents.

The observations on skeletal muscle, on various types of smooth muscle whether supplied with sympathetic or parasympathetic fibers, and on ganglion cells all point to a general rule that depriving a structure of its nerves makes it more responsive to stimulating agents. Quite possibly similar phenomena occur in the central nervous system.

SUMMARY

Evidence indicates that denervated skeletal and smooth muscles are sensitized to the natural chemical mediators of the nerve impulses which cause them to contract. Sympathetic ganglion cells may be regarded as innervated by preganglionic fibers. Acetylcholine, either injected (fig. 1) or applied locally (fig. 2), has a much greater effect in stimulating the superior cervical ganglion chronically deprived of its preganglionic innervation than the superior cervical ganglion acutely denervated.

Increased sensitiveness of the denervated structures is attributed to a greater permeability of these structures to various stimulating agents (compare figs. 1 and 2 with fig. 3).

REFERENCES

ANDERSON, H. K. J. Physiol. **33:** 414, 1905.
BROWN, G. L. AND W. FELDBERG. Ibid. **84:** 12P, 1935.
DALE, H. H. AND W. FELDBERG. Ibid. **81:** 39P, 1934.
DALE, H. H. AND H. S. GASSER. J. Pharmacol. and Exper. Therap. **29:** 53, 1926.
DUKE-ELDER, W. S. AND P. M. DUKE-ELDER. Proc. Roy. Soc. London, Series B, **107:** 332, 1931.
ELLIOTT, T. R. J. Physiol. **32:** 401, 1905.
FELDBERG, W. AND J. H. GADDUM. Ibid. **81:** 305, 1934.
FELDBERG, W., B. MINZ AND H. TSUDZIMURA. Ibid. **81:** 286, 1934.
HAMPEL, C. W. This Journal **111:** 611, 1935.
KIBJAKOV, A. W. Pflüger's Arch. **232:** 432, 1933.
LANGLEY, J. N. AND H. K. ANDERSON. J. Physiol. **19:** 131, 1895.
LICHTWITZ, L. AND C. HIRSCH. Deutsch. Arch. f. klin. Med. **99:** 125, 1910.
MELTZER, S. J. AND C. M. AUER. This Journal **11:** 28, 1904.
PEARCE, R. G. Ztschr. f. Biol. **62:** 243, 1913.
ROSENBLUETH, A. This Journal **100:** 443, 1932.
SHEN, S. C. AND W. B. CANNON. Chinese J. Physiol., 1936, in press.
SHIMIDZU, K. Arch. f. exper. Pathol. u. Pharmakol. **104:** 254, 1924.

THE ADEQUACY OF THE CHEMICAL THEORY OF SMOOTH MUSCLE EXCITATION

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In recent literature on the chemical mediation of nerve impulses in smooth muscle (see Cannon, 1933, for references) no explicit statement occurs as to whether this is the only mode of transmission possible. Tacitly, however, such an idea has probably been prevalent. Monnier and Bacq (1935) have suggested that two modes of transmission occur; the chemical, by formation of an autonomomimetic substance, and the electrical, the nerve action potential being a direct excitatory agent.

The great importance of this suggestion led us to the present study. It is our purpose to show that some experimental data are not accounted for by the dual theory of Monnier and Bacq and that an alternative interpretation, which postulates exclusively a chemical mediation, may explain the data on which Monnier and Bacq base their hypothesis.

METHODS. Cats were used under dial anesthesia (Ciba, 0.7 to 0.8 cc. per kgm. intraperitoneally). The following neuromuscular systems were studied: cervical sympathetic—nictitating membrane (n.m.), abdominal sympathetics—pilomotors of the tail (p.m.), splanchnics—stomach.

The mechanical responses of the nictitating membrane and stomach were recorded isotonically or isometrically; the erection of the pilomotors was observed directly (cf. Rosenblueth, 1932).

The electric responses were recorded as described by Lambert and Rosenblueth (1935). The stimuli employed were induction shocks from a Harvard inductorium or a Bishop balanced coil, or rectangular waves from a multivibrator circuit discharged through a transformer. The drugs used were the following: yohimbine hydrochloride (Merck); cocaine hydrochloride (Merck); adrenalin (Parke, Davis); piperidinomethyl-3-benzodioxane (933F); and diethylaminoethyloxycymene (929F). The last two drugs were kindly furnished to us by Dr. D. Bovet. All injections were made into a femoral vein.

RESULTS. *a. Yohimbine.* Injections of this drug (3 to 10 mgm. per kgm.) depress markedly the contractile action of adrenine on the n.m.; the contractions in response to stimulation of the cervical sympathetic,

on the other hand, are less impaired (fig. 1). For equal heights of responses to nerve stimulation the duration of the period of relaxation is as a rule shorter after than before yohimbine.

After an injection of cocaine (8 mgm. per kgm.), stimulation of the hepatic or cardio-accelerator nerves, which leads to liberation of sympathin E (Cannon and Rosenblueth, 1933), induces marked contractions of the n.m. Yohimbine abolishes these contractions.

The electrograms of the n.m. and the p.m. have been systematized in a previous communication from this Laboratory into three components: I, II and III (Lambert and Rosenblueth, 1935). In the present study we have been especially concerned with the components I and II in response to single shocks or to short series of stimuli at frequencies of 0.5 to 2 per

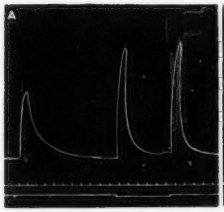

Fig. 1

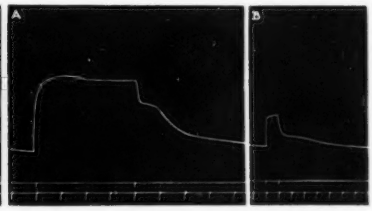

Fig. 2

Fig. 1. Dial. Cocaine. Isotonic records of the nictitating membrane (n.m.). Preganglionic stimulation. Harvard inductorium, 5 volts in the primary circuit, coil distance 7 cm. Time signal: 30 sec. intervals.

A. Before yohimbine. Responses to adrenalin (2 γ) and to a make and a break shock. B. After yohimbine (3 mgm. per kgm.). Responses to a make and a break, and three pairs of shocks and to adrenalin (20 γ).

Fig. 2. Isotonic records of the n.m. Preganglionic stimulation. Multivibrator—transformer stimuli. Time signal: 30 sec. intervals.

A. Dial. Between signals stimulation at a frequency of 30/sec. B. Dial. 933F (6 mgm. per kgm.). Between signals stimulation at a frequency of 5 per sec.

second. A study of the electrograms on repetitive stimulation at higher frequencies will be published separately (Rosenblueth, Davis and Rempel, 1936).

Yohimbine does not appreciably alter the latency of the electric responses of the n.m. or the p.m. The heights of I and II are increased. This effect is especially noticeable in the responses to the last stimuli of short series (4 to 8 shocks) at the frequencies mentioned. The duration of I is not significantly changed. The augmenting effect of yohimbine on the electrograms is more marked if cocaine (see *c* below) has been previously injected (fig. 3) than without this drug.

b. Piperidinomethyl-3-benzodioxane (933F). This drug has been studied

by Bacq and Fredericq (1935a, b) and Monnier and Bacq (1935). Its effects are in all points similar to, and usually even more striking than, those described above for yohimbine. The contractile responses of the n.m. to adrenine are considerably decreased (Bacq and Fredericq, 1935a), those to sympathin from distant sources are abolished (Bacq and Fredericq, 1935b).

The mechanical responses of the n.m. to stimulation of the cervical sympathetic are also decreased by 933F, but much less than are those to adrenine (cf. Bacq and Fredericq, 1935a). The decrement is proportional to the doses of 933F employed. We have not observed a significant increase of the mechanical responses of the n.m., whether to single shocks or to repetitive stimulation of the cervical sympathetic (cf. Monnier and Bacq, 1935).

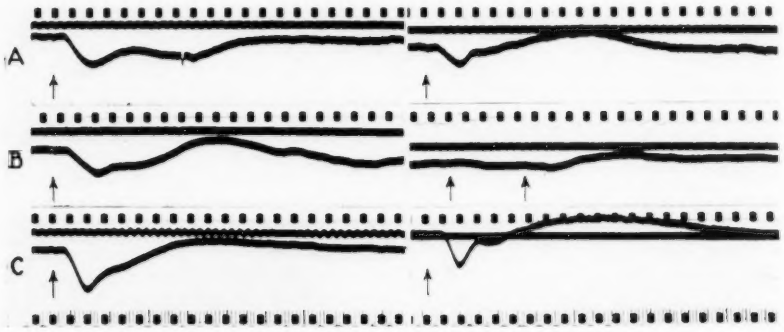

Fig. 3. Isometric mechanical (upper tracings) and electric (lower tracings) responses of the n.m. to the 1st and 3rd make shocks in short series at a frequency of 0.5 per sec. Calibration of the electric responses: 1 mv. = 27 mm. on the original 35 mm. film. Time signal: 10 msec. In this and the succeeding electrical records the arrows denote the time of application of the stimuli.

A. Before cocaine and yohimbine. B. After cocaine (8 mgm. per kgm.). C. After cocaine and yohimbine (3.5 mgm. per kgm.).

Bacq and Fredericq (1935a) reported two peculiarities of the mechanical responses of the n.m. after 933F, which Monnier and Bacq (1935) consider of great theoretical interest. The first of these peculiarities is that the relaxation curve sometimes does not decrease smoothly, but may be decomposed into two segments, one of sharp descent, then one of slower rate of decline, the latter showing occasionally even a transient rise. We have readily confirmed this observation, but find also that a similar curve of relaxation may appear in animals to which no 933F has been administered (fig. 2). Relatively high frequencies of stimulation (20 per second or more) are favorable for the appearance of the phenomenon before 933F, while

relatively slow frequencies (3 to 10 per second) are more favorable after 933F.

The second peculiarity reported by Bacq and Fredericq is that after 933F the contraction of the n.m. to a series of stimuli may not be well sustained, i.e., an initial rise is shortly succeeded by a fall to a lower level of contraction, although the stimulus is regularly applied with a frequency, e.g., of 3 per second. We have not observed this failure to maintain contraction. In no instance, whether the record was isotonic or isometric, could we detect any significant difference in this respect between the responses of the n.m. before and after 933F. As Orías (1932) has shown, when high frequencies (e.g., 40 per second) of stimulation of the pregan-

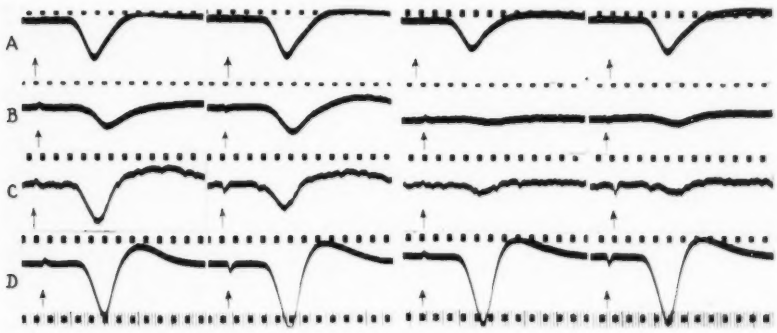

Fig. 4. Electric responses of the pilomotors (p.m.). A and B, 1 mv. = 40 mm. C and D, 1 mv. = 55 mm. Time signal: 10 msec. intervals. Series of make (m.) and break (b.) shocks applied at a frequency of 1 per sec.

A. Before cocaine and 933F. 1st and 2nd m. and b. B. After cocaine (8 mgm. per kgm.). 1st and 2nd m. and b. C. As in B, amplification increased. 1st and 4th m. and b. D. After cocaine and 933F (3 mgm. per kgm.). 1st and 4th m. and b.

glionic fibers are employed, the normal n.m. fails to sustain its contraction, and slowly and smoothly falls to lower levels after relatively long periods of stimulation (2 minutes or more). We have observed precisely the same phenomenon after 933F, but have not been able to detect any decline of the responses with frequencies lower than 10 per second. A possible explanation for this discrepancy between Bacq and Fredericq's observations and ours will be suggested in the discussion.

As regards the electric responses of the n.m. and the p.m., the component I is usually larger after 933F than before injection of the drug, and sustains its amplitude better when a series of shocks is applied at a slow frequency. The component II is also sometimes increased by 933F. The duration of I is not significantly altered by this drug (see table 1). Mon-

nier and Bacq (1935) reported an increase in magnitude and a shortening of the duration of the "action potentials" of the n.m. after 933F. They did not discriminate, however, between the components I and II (see Lambert and Rosenblueth, 1935, for a criticism of these undiscriminating statements about "action potentials"). Figures 4 and 5 illustrate typical effects of 933F.

c. Cocaine. The increasing and lengthening effects of cocaine on the mechanical responses of the n.m. to adrenine, sympathin E from distant sources, and cervical sympathetic stimulation have been previously reported (Rosenblueth and Cannon, 1932; Rosenblueth and Rioch, 1933a).

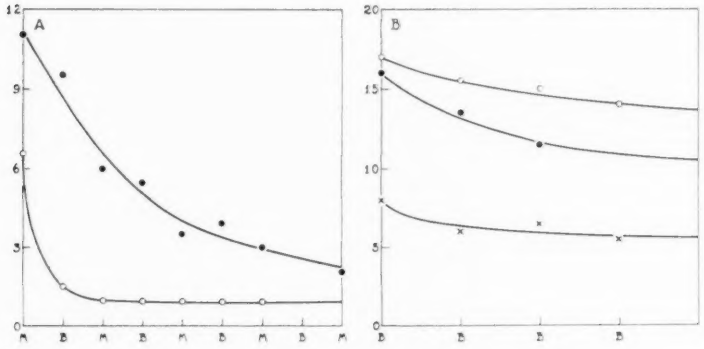

Fig. 5. Magnitude of the electric responses of the n.m. to series of stimuli at a frequency of 0.8 per sec. Ordinates: height of I in mm. in the records. Abscissae: successive shocks (M, makes; B, breaks). The same amplification was used throughout.

A. Dots: before cocaine. Circles: after cocaine (8 mgm. per kgm.) B. Dots: before 933F and cocaine. Circles: after 933F (3 mgm. per kgm.). Crosses: after 933F and cocaine (8 mgm. per kgm.).

Monnier and Bacq (1935) state that the "action potentials" of the n.m. become longer and disappear rapidly on a series of stimuli after cocaine. Figures 3 and 4 show a marked decrease of I and II in the n.m. and p.m. on repetitive stimulation (see also fig. 5). The duration of I, on the other hand, is not significantly altered by cocaine (cf. table 1).

When cocaine is injected after 933F the breaks in the relaxation curves described above (*b*) become strikingly more prominent.

d. Adrenine. Injections of adrenine (1 to 20 γ) considerably depress the electric responses, I and II, of both the n.m. and the p.m. on stimulation of their nerve supply. This depression is transient and follows a time course comparable to that of other effects of adrenine, e.g., the contractions of the n.m. The depression is increased by cocaine and decreased to

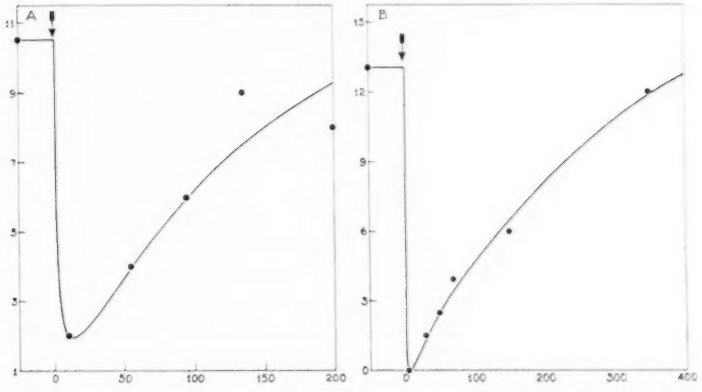

Fig. 6. Action of adrenine on the electric responses to single shocks of the n.m. and p.m. Ordinates: magnitude of I in mm. in the records. Abscissae: seconds.

A. Dial. N.m. At arrow, adrenalin (40 γ) injected intravenously. B. Dial. Cocaine. P.m. At arrow adrenalin (20 γ) injected intravenously.

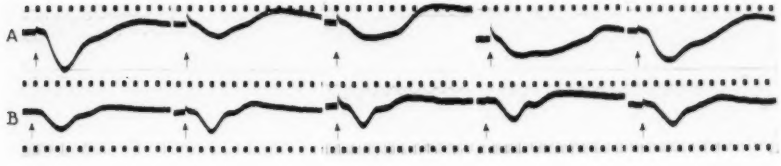

Fig. 7. Electric responses of the n.m. to single shocks.

A. Before 933F. 1 mv. = 55 mm. The first record was obtained before the injection of adrenalin (40 γ). The succeeding records were taken 30, 50, 85 and 240 sec. after the injection. B. After 933F (2.5 mgm. per kgm.). 1 mv. = 27 mm. The first record was obtained before the injection of adrenalin (40 γ). The succeeding records were taken 30, 70, 120 and 180 sec. after the injection.

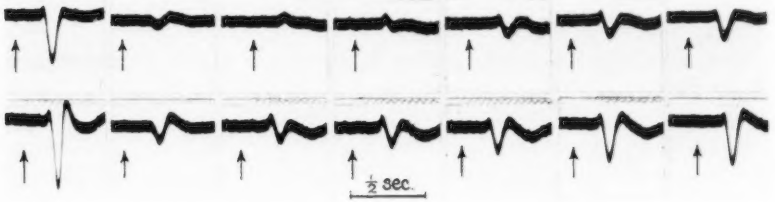

Fig. 8. Electric responses of the p.m. to make (upper records) and break (lower records) shocks. The first records (upper and lower) were obtained before the injection of adrenalin (20 γ). The succeeding records were taken 15, 45, 60, 115, 180 and 300 sec. after the injection. The tension of the string was initially 60 mm. per mv. At the end of the series it was 50 mm., which accounts partly for the failure of the last responses to attain the magnitude of the first.

practical disappearance by yohimbine and 933F. Figures 6, 7 and 8 illustrate these effects.

c. Preganglionic chronic denervation of the n.m. After section of the cervical sympathetic chain and degeneration of the preganglionic fibers, the n.m. becomes gradually sensitized to adrenine—i.e., the responses of the chronically decentralized n.m. are markedly greater than those of the acutely decentralized one (Elliott, 1905; Hampel, 1935).

This sensitization, similar in quality to that obtained by injections of cocaine or by chronic postganglionic denervation, suggested the interest of investigating the responses of the preganglionically denervated n.m. (p.d.n.m.) on stimulation of its postganglionic nerve supply. Accordingly, in several cats the right cervical sympathetic chain was severed under aseptic conditions, and 2 to 40 days later electrodes were placed on

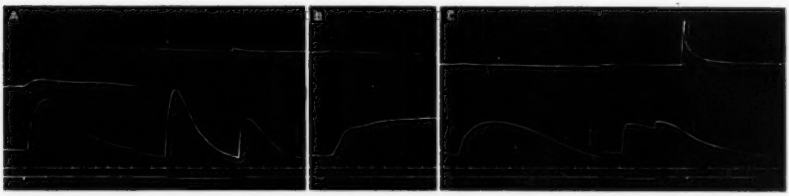

Fig. 9. Right cervical sympathetic (preganglionic) cut 9 days before. Dial. Left cervical sympathetic cut. Upper tracings: left n.m. Lower tracings: right n.m. Time signal: 30 sec. intervals.

A. Before 933F. Responses to adrenalin (2 γ) and to a make and a break induction shock applied to both postganglionic nerves (coil distance 4 cm.). B. Injection of 933F (3 mgm. per kgm.). C. After 933F. Responses to adrenalin (20 γ), to a make and a break induction shock applied to both postganglionic nerves (coil distance 4 cm.), and to two pairs of shocks applied to the left postganglionic nerve.

the right postganglionic fibers and on the left pre- or postganglionic fibers, and the mechanical and electric responses of both n.ms. were studied comparatively.

The mechanical responses of the p.d.n.m. are strikingly bigger and longer than those of the normal control. The difference is obvious when even single maximal shocks are applied to both sides (fig. 9A). Injections of 933F elicit a persistent moderate contracture of the p.d.n.m., absent on the normal side (fig. 9B). Stimulation of the nerve supply now evokes smaller responses than before 933F, larger still, however, than on the normal control (fig. 9C). The contractions of the p.d.n.m. are longer after than before 933F, in contrast with the contractions of the normal n.m., whose duration is not significantly or consistently changed by 933F (cf. Bacq and Frederieq, 1935a).

Injections of cocaine, before or after 933F, induce usually slight contractures of the p.d.n.m. Stimulation of the postganglionic supply after 933F and cocaine causes higher and shorter responses than before cocaine. All these results occurred after ligating the adrenals and crushing the right superior cervical ganglion, showing that these structures were not concerned with the effects recorded.

The electric responses of the p.d.n.m. resemble those of the cocainized n.m. (see *c* above), being smaller than on the normal control side and declining rapidly after a short series of stimuli (fig. 10). Injections of 933F and of cocaine have effects on the electrograms of the p.d.n.m. similar to those exerted on the normal n.m. (sections *b* and *c*).

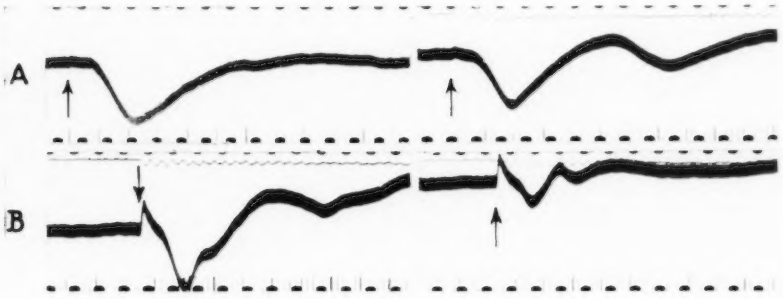

Fig. 10. Right cervical sympathetic (preganglionic) cut 40 days before. Electric responses of the two n.ms. 1 mv. = 36 mm. Stimulation at a frequency of 0.5 per sec. Time signal: 10 msec.

A. Left n.m. Responses to the 1st and 2nd make shocks applied to the preganglionic nerve. B. Right n.m. Responses to the 1st and 2nd make shocks applied to the postganglionic nerve.

f. Diethylaminoethyloxycymene (929F). The action of this drug on the inhibitory effects of adrenine and splanchnic stimulation on gastric peristalsis was studied. Bovet and Maderni (1933) reported a suppression of the inhibition of the intestine of the dog by adrenine after injections of 10 mgm. per kgm. of 929F. In the present study inhibition by adrenine of the cat's gastric peristalsis was considerably attenuated, though not abolished by this drug (6 to 20 mgm. per kgm.). Inhibition by stimulation of the splanchnics, on the other hand, was only slightly decreased after 929F. Figure 11 illustrates this relative differential effect of 929F.

Discussion. The theory of a dual mode of excitation of smooth muscle as stated by Monnier and Baeq (see introduction) does not discriminate between the transmission of the nerve impulse and the initiation of contraction. According to the chemical theory (see Cannon and Rosenblueth, 1933), the nerve action-potential is the stimulus for the liberation of the

chemical mediator; this mediator combines with the receptive substance; the compound thus formed activates contraction. The potentials I, II and III may be correlated with some of these steps (see Rosenblueth, Davis and Rempel, 1936). Monnier and Bacq do not make clear what steps they assume when they postulate the electrical transmission. It appears, however, that the sequence they conceive is the following: nerve action potential → muscle action potential (I or II?, see Lambert and Rosenblueth, 1935) → slow depolarization (III in our nomenclature, cf. Bacq and Monnier, 1935) → contraction.

A clear statement of all the assumptions made is indispensable for a criticism of any theory. Since Monnier and Bacq have not made their assumptions explicit, we shall have to base our discussion on the interpretation just developed.

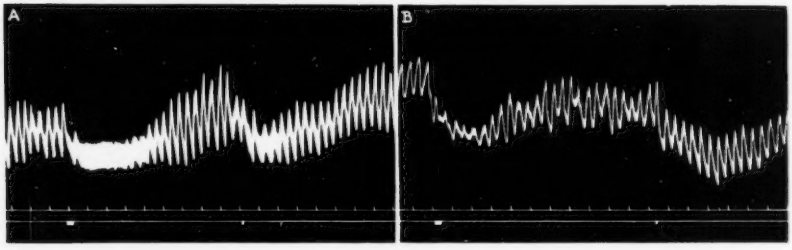

Fig. 11. Urethane (1 gram per kgm.). Effects of stimulating the right major splanchnic nerve (tetanizing frequency, coil distance 10 cm.) and of injecting adrenalin (5 γ) on gastric peristalsis. Time signal: 30 sec. intervals.
A. Before 929F. B. After 929F (6 mgm. per kgm.).

The dual theory does not account reasonably for the following experimental data. When cocaine is injected after yohimbine or 933F the mechanical responses of the n.m. to a given dose of adrenine, although increased by cocaine, may remain considerably reduced as compared to those obtained before 933F. Monnier and Bacq postulate that the action of the chemical mediator is depressed by 933F as much as that of adrenine; the responses to nerve stimulation, therefore, should be due in these conditions mainly to the action potentials. But, while the mechanical responses are increased by cocaine, the action potentials, both I and II, are in fact reduced (section *c*).

Furthermore, Bacq and Fredericq (1935a) report that, when cocaine is injected after 933F, the increase of the mechanical responses of the n.m. to stimulation of the cervical sympathetic is quantitatively identical with the increase of the responses to adrenine. Since cocaine reduces the action potentials, however, this identity of increment fails to be explained

by the assumption that, before cocaine, the responses to the nerve impulses occur as a direct effect of the action potentials.

After ergotoxine, the electric responses of the p.m. are practically normal (Rosenblueth, Leese and Lambert, 1933), while the mechanical responses disappear. The muscles do not go into contracture, for the hairs do not erect. There is no ready explanation for the failure of the action potentials to evoke contraction, if they are direct physiological stimuli.

The reduction of the action potentials by adrenine (section *d*) is again a fact difficult to reconcile with the dual theory. General biological principles would lead us to expect that two physiological stimuli which act in the same direction would be synergistic, not antagonistic.

The main argument offered by Monnier and Bacq for their dual theory is the paralyzing effect of 933F on the responses of the n.m. to adrenine and its relatively minor action on the responses to stimulation of the sympathetic supply. The effects of 929F on the inhibition of gastric peristalsis are similar, however, to those of 933F on the n.m. (section *f*). If we were then to apply a similar reasoning we should come to the conclusion that action potentials in the stomach (which have not been demonstrated in response to splanchnic stimulation) are partly responsible for the inhibition. Aside from the improbability of such a view, the explanation would not satisfy Monnier and Bacq's specification that the action potentials are only present and act as effective stimuli in non-iterative systems—i.e., systems which respond to single volleys of nerve impulses—for the splanchnic-stomach system may be iterative, since no responses have been demonstrated to single stimuli.

When the sympathetic supply of the n.m. or p.m. is stimulated at frequencies higher than 15 per second, after a few shocks have been applied no action potentials are demonstrable (cf. Rosenblueth, Davis and Rempel, 1936). This effect occurs also after 933F. Since in this case, according to Monnier and Bacq, the sole stimulus effective is the action potential, the n.m. should relax after a few stimuli, but it does not; indeed, the tension is well maintained throughout stimulation (section *b*).

Stress is laid by Monnier and Bacq on the shortening of the mechanical responses of the n.m. after 933F as an argument in favor of the rôle of the action potential as a stimulus. But in the present study we failed to obtain consistently this shortening and found instead 933F lengthening the twitches of the p.d.n.m. (fig. 9).

Other arguments offered by Monnier and Bacq in favor of their dual theory are the following. They attribute the complex relaxation curves they observed after 933F (see section *b*) to an initial rapid subsidence of the part of the contraction due to the action potentials, and the slow protracted component to the more persisting effects of the chemical mediator. We have pointed out (fig. 2) that a complex relaxation curve may occur

without 933F. Such a relaxation should therefore not be attributed to the hypothetical depression of the action of the chemical mediator by 933F. Furthermore, when these complex relaxation curves appear, with or without 933F, there are not only changes in the rate of a continuous relaxation, but the sharp initial fall may be succeeded by a delayed rise in the mechanical record. The explanation of Monnier and Bacq would then lead us to postulate that larger amounts of chemical mediator are produced long after the nerve impulses have ceased, a view which is quite unlikely.

Monnier and Bacq attribute to Wedensky inhibition the decline in the mechanical response during continued stimulation at low frequencies (3

TABLE 1

Average latencies and durations of the ascending phase of potential I in milliseconds for the nictitating membrane and pilomotors

The total duration was not measured since it is usually impossible to determine the end of I, as II may begin during I. The figures are given only for the cases in which a sufficient number of observations were made to justify drawing an average. The margin of error of these averages is approximately 5 and 10 msecs. for the latencies and durations, respectively. Since the latency of the pilomotors varies with the distance of the recording muscles to the stimulating electrodes, it was only measured in experiments involving standard uniform conditions.

	NICTITATING MEMBRANE				PILOMOTORS	
	Preganglionic stimulation		Postganglionic stimulation			
	Latency	Ascending phase	Latency	Ascending phase	Latency	Ascending phase
Normal	34	54	28	33	120	71
After 933F	38	60	28	36	123	64
After cocaine	41	69			127	76

to 10 per second) after 933F which Bacq and Fredericq (1935a) reported. They next argue that Wedensky inhibition is a proof of an electric transmission. We have been unable to confirm this decline at low frequencies (p. 418). Bacq and Fredericq do not mention any precautions to eliminate movements of the eye (see Rosenblueth and Bard, 1932) which may occur and artifactually alter the isotonic records of the n.m. It is possible that such an extraneous factor may have been responsible for the quite atypical record they reproduce (fig. 6 in their paper).

A final argument adduced by Monnier and Bacq in support of their dual theory is that 933F, which would increase and shorten the action potentials of the n.m., favors the appearance of mechanical responses to single shocks applied to the cervical sympathetic. We have failed to con-

firm any significant shortening of I after 933F. Indeed, our data (table 1) show the fallacy of deriving any conclusions from the duration of the action potentials, when the preganglionic fibers are stimulated, for the ganglion obviously introduces a temporal dispersion of the efferent postganglionic impulses, as shown by the significant differences in the durations of the responses to pre- and to postganglionic stimulation. We have also failed to confirm the favorable action of 933F on the appearance of responses to single shocks. Invariably all the mechanical responses, whether to single or to repetitive stimuli, applied to either the preganglionic or the postganglionic fibers, were smaller after any dose of 933F than before the injection of the drug (section *b*).

The latencies of the electric responses of the n.m. and the p.m. after the nerve impulses have reached the muscles are at least 20 and 60 msec., respectively (Rosenblueth, Davis and Rempel, 1936), and are not significantly altered by 933F (table 1). These neuromuscular delays can not be explained reasonably if the nerve action potential directly activates the muscular electric response, while they are readily accounted for if they are assumed to represent the period of production of the mediator (Rosenblueth, Davis and Rempel, 1936).

Having thus shown that the dual theory of Monnier and Bacq fails to account reasonably for several experimental data, it remains to inquire whether these and other data available may be explained exclusively in terms of a chemical mediation.

The following assumptions are generally accepted: that the permeability of cells is inversely proportional to the polarization; and that the spike action potential (I in our nomenclature) is directly proportional to the polarization (cf. Lillie, 1932; Dubuisson, 1935). A histological fact of capital importance from the standpoint of this discussion is that autonomic nerves distributed to smooth muscle may frequently end inside, not outside the muscle cells (see Cannon, 1933, for references).

On the basis of these data and assumptions the experimental facts may be explained as follows. Drugs which make certain smooth muscle cells less permeable will antagonize the responses to adrenine or other substances injected into the blood stream, because these substances will not be able to enter the muscle cells, therein to become effective. Stimulation of the autonomic nerve supply, on the other hand, may still be capable of eliciting a response, when the chemical mediator is produced inside the cells. The actions of yohimbine and 933F, discussed above, may thus be accounted for by a decrease in permeability and an increase in polarization. This view is in accord with the explanation offered by Dale and Gaddum (1930) for the similar effects of atropine. Indeed, when injected adrenine fails to evoke contractions of the n.m. after 933F, there is no evidence that the hormone reaches the muscle cells. A decreased per-

meability of some blood vessels might oppose the diffusion of the hormone into some tissues.

The other actions of 933F on the n.m. are in agreement with this explanation. Thus the spike action-potentials (I) of the n.m. and the p.m. are enhanced (section *b*). Similarly yohimbine increases the spike potentials (section *a*).

It has been suggested (Rosenblueth and Morison, 1934) that cocaine increases the responses of smooth muscles to adrenine and sympathin because it makes the cells more permeable to these substances. In keeping with this suggestion is the decrease of the spike potentials of the n.m. and p.m. (section *c*), which occurs after cocaine.

The increased responses of the n.m. to adrenine, drugs and ions, which appear after denervation (see Hampel, 1935, for references) could also be explained as due to an increased permeability of the denervated cells. The decrease of the spike potentials in the p.d.n.m. (section *e*) is in accord with this suggestion.

The decrease of the action potentials of both the n.m. and the p.m. induced by adrenine (section *d*) deserves special consideration. In the n.m. adrenine evokes a contraction; the responses to the nerve impulses and those to adrenine, however, sum freely (Rosenblueth and Rioch, 1933a). In the pilomotors, on the other hand, adrenine does not as a rule evoke contraction, as is well known, although, after cocaine, adrenine (in the doses employed in these experiments) evokes slight transient erections of the hairs (unpublished observations). These data lead us to infer that the reduction of the action potentials of the n.m. is not a consequence of the contractions (cf., p.m. without cocaine). We may then assume that adrenine, like cocaine and denervation, depolarizes smooth muscle, making it more permeable. Such an assumption furnishes an explanation for the sensitization to injected adrenine of the dilator muscle in the iris when adrenine is instilled in the conjunctiva (Schlossberg, 1932; and Sawyer and Schlossberg, 1933).

This hypothesis affords also an explanation for the behavior of the action potentials of the n.m. and p.m. on repetitive stimulation (cf. Rosenblueth, Davis and Rempel, 1936). As the frequency of stimulation increases, the action potentials of both n.m. and p.m. decrease rapidly after a few shocks have been applied to the nerve. This decrease would be a consequence of the depolarization caused by the locally liberated adrenine-like chemical mediator (see Cannon and Rosenblueth, 1933).

That adrenine may influence the polarization of cell membranes has been shown by Monnier and Dubuisson (1934), and Osterhout and Hill (1935). The prevention of the decreasing effect of adrenine by 933F (section *d*) is readily explained by the polarizing action of the latter (see above).

In table 2 the results discussed in this paper are summarized. If we interpret the changes in the mechanical responses to adrenine as due to changes in permeability, as suggested above, we may infer inverse changes in polarization (table 3). The correspondence between the changes in the action potentials (table 2) and the inferred changes in polarization (table 3) support the assumption that the former may be used as an index of the latter.

The increased mechanical twitches of the p.d.n.m. as compared to the normal n.m. (section *e*) may be explained by either of the two following

TABLE 2

Summary of results

The signs have the following significance: +, increase; −, decrease; 0, disappearance.

	MUSCLE ACTION POTENTIALS (I AND II)		MECHANICAL RESPONSE TO NERVE STIMULATION		MECHANICAL RESPONSE TO ADRENINE	
	N.M.	P.M	N.M	P.M.	N.M.	P.M
Yohimbine	+	+	−		0	
933F	+	+	−		0	
Cocaine	−	−	+		+	+
Adrenine	−	−	+		+	
Preganglionic denervation	−		+		+	
Ergotoxine	0	±	0	0	0	

TABLE 3

	PERMEABILITY	POLARIZATION
Yohimbine	−	+
933F	−	+
Cocaine	+	−
Adrenine	+	−
Preganglionic denervation	+	−

mechanisms. Assuming that denervation has increased the permeability of the muscle (see above) and bearing in mind the histological evidence that only a slight fraction of the total number of cells in the muscle are innervated (see Cannon, 1933, for references), we may conclude that the diffusion of the chemical mediator from the innervated cells to the non-innervated neighboring elements (cf. Rosenblueth and Rioch, 1933b) would occur more readily after than before denervation. For the combination of the mediator M with the receptive substance E to form the active compound ME (Cannon and Rosenblueth, 1933), the greater diffusion after denervation is tantamount to an increase of the E available

for combination with M. According to the mass-action law the amounts of ME formed for the same amounts of M liberated would then be greater after than before denervation. The second assumption which may account for the greater twitches of the p.d.n.m. is that larger amounts of M are liberated per nerve impulse. It is likely that both factors play a rôle. Finally, since the responses are more prolonged after denervation, it is probable that the denervated muscle is less capable of destroying adrenine (M) than the normal tissue. Similarly, cocaine may prevent the destruction of adrenine, hence the more prolonged responses after this drug (cf. Rosenblueth and Morison, 1934; Bacq and Bovet, 1935).

SUMMARY

The action of yohimbine, 933F, cocaine and adrenine on the electric and mechanical responses of the nictitating membrane and tail pilomotors of the cat to stimulation of their sympathetic nerve supply is described (sections *a*, *b*, *c* and *d*). The effects of chronic preganglionic denervation of the nictitating membrane on its electric and mechanical responses are reported (section *e*). The influence of 929F on the inhibition of gastric peristalsis by adrenine and splanchnic stimulation is analyzed.

From this evidence it is concluded that certain substances (e.g., cocaine and adrenine) and chronic decentralization depolarize smooth muscle, rendering it more permeable (pp. 427–428), while other substances (e.g., yohimbine and 933F) increase the polarization and decrease the permeability (pp. 426–428).

It is further concluded that there is no electrical transmission of the nerve impulses to smooth muscle (p. 426) and that the theory of chemical mediation accounts satisfactorily for the data available.

REFERENCES

Bacq, Z. M. and D. Bovet. Arch. Internat. Pharmac. Thérap. **50:** 315, 1935.
Bacq, Z. M. and H. Fredericq. Arch. Internat. Physiol. **40:** 454, 1935a.
Ibid. **41:** 334, 1935b.
Bacq, Z. M. and A. M. Monnier. Ibid. **40:** 467, 1935.
Bovet, D. and P. Maderni. Compt. Rend. Soc. Biol. **114:** 980, 1933.
Cannon, W. B. Science **78:** 43, 1933.
Cannon, W. B. and A. Rosenblueth. This Journal **104:** 557, 1933.
Dale, H. H. and J. H. Gaddum. J. Physiol. **70:** 109, 1930.
Dubuisson, M. Arch. Internat. Physiol. **41:** 511, 1935.
Elliott, T. R. J. Physiol. **32:** 401, 1905.
Hampel, C. W. This Journal **111:** 611, 1935.
Lambert, E. F. and A. Rosenblueth. Ibid. **114:** 147, 1935.
Lillie, R. S. Protoplasmic action and nervous action. Chicago, 1932.
Monnier, A. M. and Z. M. Bacq. Arch. Internat. Physiol. **40:** 485, 1935.
Monnier, A. M. and M. Dubuisson. Ibid. **38:** 180, 1934.
Orías, O. This Journal **102:** 87, 1932.
Osterhout, W. J. V. and S. E. Hill. Proc. Soc. Exper. Biol. Med. **32:** 715, 1935.

ROSENBLUETH, A. This Journal **102:** 12, 1932.
ROSENBLUETH, A. AND P. BARD. Ibid. **100:** 537, 1932.
ROSENBLUETH, A. AND W. B. CANNON. Ibid. **99:** 398, 1932.
ROSENBLUETH, A., H. DAVIS AND B. REMPEL. Ibid. **116:** 388, 1936.
ROSENBLUETH, A., C. LEESE AND E. LAMBERT. Ibid. **103:** 659, 1933.
ROSENBLUETH, A. AND R. S. MORISON. Ibid. **109:** 209, 1934.
ROSENBLUETH, A. AND D. MCK. RIOCH. Ibid., **103:** 681, 1933a.
Ibid. **106:** 365, 1933b.
SAWYER, M. MACK. AND T. SCHLOSSBERG. Ibid. **103:** 153, 1933.
SCHLOSSBERG, T. Ibid. **102:** 71, 1932.

EXPERIMENTS ON INTACT AND ADRENALECTOMIZED DOGS SUBJECTED TO SODIUM AND CHLORIDE DEPLETION BY INTRAPERITONEAL INJECTIONS OF GLUCOSE

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This communication is concerned with the effect of adrenal cortical hormone upon the internal shift of body water induced by sodium and chloride depletion in intact and adrenalectomized dogs. The work is based upon the observations of Darrow and Yannet (1935) and Gilman (1934). According to their interpretations, the altered water distribution induced in normal dogs by intraperitoneal glucose injections followed by paracentesis, is due to a shift of extracellular water into the body cells, producing dehydration of extracellular fluids. This water shift is a result of depletion of extracellular electrolytes unaccompanied by loss of body water, and the concomitant reduction in the osmotic pressure of the extracellular fluid. A direct consequence of this upset in osmotic equilibrium and shift of fluids into intracellular compartments, is a great decrease in blood volume, arterial pressure and increase in hemoconcentration. The fluids so shifted are osmotically held within the cells owing to the higher concentration of intra- to extracellular electrolyte and are thereby rendered unavailable (only temporarily so in the dog with intact adrenal glands, according to our data) to the animal in so far as a reverse transfer across the cell membrane for blood diluting purposes is concerned.

We are particularly concerned in the following experiments with the action of the adrenal cortical hormone in releasing the osmotically bound intracellular fluids in the adrenalectomized dog lacking reserve hormone, and the rôle which the intact adrenal glands play in the sodium and chloride depleted animal.

MATERIAL AND METHODS. Strong healthy dogs, table trained for blood pressure determinations were used. Blood samples were taken directly from the femoral artery and the following methods employed: Blood pressure, Parkins (1934); serum sodium, Butler and Tuthill (1931); serum chlorides, Van Slyke and Sendroy (1923) as modified by Eisenman (1929); hemoglobin, Newcomer; cell volume, sealed capillary tubes centrifuged at

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20,000 times gravity; the heights of the columns of blood and cells were measured with calipers. The animals were injected intraperitoneally with either 100 cc. or 50 cc. of 5.5 per cent glucose per kilogram of body weight, the amount depending upon the nature of the experiment. After an interval a paracentesis was performed and an amount of fluid equivalent to that injected was withdrawn using the method previously described (Parkins, Taylor and Swingle, 1935). The ascitic fluid was analyzed for sodium and chloride and the blood pressure again determined. In the hemorrhage experiments which are similar to those of Gilman (1934), the dogs were bled 10 cc. per kilogram over a period of 30 to 60 minutes and the arterial pressure subsequently recorded every few hours.

Effect of sodium and chloride depletion in the intact animal. Table 1 illustrates depletion of the body sodium and chloride following intraperitoneal glucose injection.

Within a short period, 3 to 5 hours in these experiments, approximately the same quantity of fluid can be recovered from the peritoneal cavity as originally injected. However, it now contains large quantities of sodium and chloride, etc., and is apparently similar to extracellular fluid.

It is a curious fact, first noted by Darrow and Yannet (1935), that despite the evident dehydration, these dogs do not drink water during the first 12 to 18 hours after paracentesis.

Blood changes. Table 2 shows the changes in serum sodium and chloride and hemoconcentration resulting from the shift of body fluids from blood stream and extracellular compartments to the tissue cells owing to the decreased osmotic pressure of the extracellular fluids. This is assumed to be primarily a result of sodium depletion, since sodium is the chief cation of the extracellular fluids.

Effect of hemorrhage upon blood pressure following sodium and chloride depletion. Loss of ability to dilute the blood, i.e., maintain the normal fluid distribution, is one of the outstanding features of the adrenalectomized dog. The animal is thus rendered extremely sensitive to small blood loss (Swingle, Pfiffner, Vars and Parkins, 1934a) and a slight hemorrhage which in the presence of intact adrenals would be negligible, leads to serious consequences. The entire loss must be borne by the circulating volume alone since compensation by shift of fluid to the blood stream does not occur in the absence of the hormone. The essential data relating to sensitivity of the intact dog to slight hemorrhage after sodium and chloride depletion by glucose injections are shown in table 3. We can confirm Gilman's (1934) observations that normal dogs subjected to these procedures are sensitive to small blood loss.

The animals were bled 10 cc. per kilogram of body weight, the blood being taken from the femoral artery. It will be noted that the blood pressure fell to shock levels. Similar sized, intact dogs, not depleted of so-

dium and chloride require a far greater loss of blood for equivalent reduction in arterial pressure. However, we are unable to confirm Gilman's statement that the sodium- and chloride-depleted dog subjected to slight

TABLE 1

Sodium and chloride depletion by intraperitoneal injection of isotonic glucose

DOG NUMBER	WEIGHT	GLUCOSE INJECTED	FLUID RECOVERED	TIME INJECTION TO PARACENTESIS	SODIUM IN ASCITIC FLUID	TOTAL SODIUM REMOVED	CHLORIDE IN ASCITIC FLUID	TOTAL CHLORIDE REMOVED
	kgm.	*cc.*	*cc.*	*minutes*	*m.-eq./l.*	*m.-eq.*	*m.-eq./l.*	*m.-eq.*
1	15.1	1,500	1,525	225	93.6	143.2	86.6	132.1
2	10.0	1,000	950	250	104.0	98.8	104.0	98.8
3	11.4	1,140	1,190	255	93.7	111.5	83.2	99.0

TABLE 2

Blood changes following sodium and chloride depletion by intraperitoneal glucose

DOG NUMBER	SERUM SODIUM		SERUM CHLORIDE		Hb		CELL VOLUME	
	Normal	After paracentesis	Normal	After paracentesis	Normal	After paracentesis	Normal	After paracentesis
	m.-eq.	*m.-eq.*	*m.-eq.*	*m.-eq.*	*grams per cent*	*grams per cent*	*per cent*	*per cent*
1	148.5	130.0	115.8	95.4	13.4	18.4	40.7	54.1
2	143.2	129.2	113.8	96.1	15.7	24.0	48.0	62.3
3	144.0	123.5	110.8	87.8	15.4	23.4	43.5	65.5

TABLE 3

Effect on blood pressure of altered water distribution due to sodium and chloride depletion and subsequent hemorrhage

DOG NUMBER	BLOOD PRESSURE			HEMORRHAGE	DURATION OF HEMORRHAGE	BLOOD PRESSURE 48 HOURS AFTER HEMORRHAGE	REMARKS
	Normal	After paracentesis	After hemorrhage				
	mm. Hg	*mm. Hg*	*mm. Hg*	*cc. per kgm.*	*minutes*		
1	108	82	66	9.3	50	104	Normal. 24 hours
2	106	86	56	10.0	50	108	Appears normal
3	110	60	47	10.0	58	100	Appears normal
4	100	64	54	6.5	30	98	Normal. 24 hours
5	98	78	50	10.0	60	94	Appears normal

hemorrhage (5.6–7.4 cc. per kgm. in his experiments) usually dies unless injected with salt. He assumes that the sodium- and chloride-depleted dog is comparable to the adrenalectomized animal not receiving extract. However, it has been our experience that depletion of sodium and chloride

in dogs possessing adrenal glands induces merely a *temporary loss* of *diluting ability* by shifting the extracellular fluids to intracellular spaces owing to fall in osmotic pressure of extracellular fluid. The normal dog invariably spontaneously recovers from the "depletion" plus hemorrhage of 10 cc. per kilogram. The adrenalectomized dog, equally healthy and vigorous lacks diluting ability, and dies following an intraperitoneal injection of only 50 cc. of glucose per kilogram of body weight, unless cortical hormone is administered or concentrated salt injected.

Effect of intraperitoneal glucose (50 cc. per kgm.) on healthy vigorous adrenalectomized dogs receiving maintenance doses hormone, similar animals primed with large doses hormone, and the intact dog. The significant data from this series of experiments (14 experiments) are given in table 4. The adrenalectomized dog on a maintenance dose of hormone is extremely sensitive to glucose injections. The arterial pressure steadily falls, the blood concentrates, and the typical picture of dehydration and shock appears. Such animals die unless injected with adequate amounts of cortical hormone. (Table 4A.) The particular animal referred to in the table was revived from shock and collapse with extract. No food was given for four days but water was allowed *ad libitum*. During this interval the serum sodium and chloride remained at the levels observed when the animal was prostrate, but all symptoms disappeared and normal activity and vigor returned. At the end of the four days of starvation the animal was again placed on the usual laboratory diet; the serum electrolytes slowly rose to their normal levels over a period of several days and all evidence of hemoconcentration disappeared.

Table 4B shows the effect of priming the adrenalectomized dog with large doses of hormone before intraperitoneal glucose administration. Three hours previous to receiving the glucose, the animal was injected with 3 cc. of cortical hormone per kilogram of body weight. This is the usual resuscitation dose used when the animals are in collapse and shock. The data reveal clearly that animals so treated react to the glucose injections in a manner very similar to that of the dog possessing adrenal glands. Both types of animal show hemoconcentration and some decline of blood pressure. (Table 4C.) The animals primed with hormone quickly dilute their blood by redistributing the body fluids and recover. The adrenalectomized dog on maintenance doses of hormone must receive additional hormone to dilute and return to normal. Compare A, B, C and D, table 4.

It will be observed that in the experiments on priming and similar experiments on the intact dog, table 4B and C, the injected glucose was not withdrawn but left within the peritoneal cavity. In the following experiment (table 4D) the adrenalectomized animals were subjected to the same depletion procedure except that priming doses of extract were not given. The animals were on the usual maintenance dose of hormone,

TABLE 4

Changes in serum sodium, chloride, arterial pressure and hemoconcentration in healthy vigorous adrenalectomized and unoperated dogs following intraperitoneal injection of isotonic glucose (50 cc. per kgm.)

DATE	TIME	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	Hb	CELL VOLUME	REMARKS
A. Adrenalectomized. Dog 8♀—8.4 kgm. Maintenance hormone. Paracentesis								
	hours	*mm. Hg*		*m.-eq.*	*m.-eq.*	*grams*	*per cent*	
12/19/35	11:30 a.m.	96	68	138.8	110.0	12.5	36.4	Normal. Glucose injected
12/19/35	10:15 p.m.	50	160	129.1	97.8	17.5	50.4	Collapse. Extract injected
12/22/35	11:15 a.m.	88	88	130.6	100.4	16.0	40.5	Active. No food
12/24/35	10:00 a.m.	92	84	127.1	101.4	15.2	37.5	Normal. No food
1/ 2/36	10:30 a.m.	95	80	135.4	114.2	13.5	40.6	Normal. Full ration
B. Adrenalectomized. Dog 8♀—7.9 kgm. Primed with hormone. No paracentesis								
12/ 2/35	1:00 p.m.	94	80	139.0	114.0	9.9	36.6	Normal. Glucose injected
12/ 2/35	4:00 p.m.	92	124	127.4	101.2	10.9	39.1	No symptoms
12/ 2/35	7:00 p.m.	78	136	125.3	100.4	11.8	41.2	No symptoms. Hormone injected
12/ 2/35	10:00 p.m.	78	130	128.1	100.2	11.3	40.0	No symptoms
12/ 3/35	11:00 a.m.	90	88	131.3	101.8	10.7	34.6	No symptoms
12/ 4/35	11:00 a.m.	92	84	137.0	112.2	8.9	29.8	No symptoms
12/ 5/35	3:00 p.m.	93	76	138.2	114.8	9.5	30.0	No symptoms
C. Unoperated. Dog 6♂—13.4 kgm. No paracentesis								
12/ 4/35	10:00 a.m.	106	72	140.8	113.2	15.1	45.4	Normal. Glucose injected
12/ 4/35	2:15 p.m.	88	100	133.6	102.4	19.0	58.0	No symptoms
12/ 4/35	5:15 p.m.	90	112	132.3	99.2	19.5	61.2	No symptoms
12/ 4/35	8:15 p.m.	102	116	134.6	101.6	17.9	55.7	No symptoms
12/ 5/35	10:00 a.m.	110	92	138.7	107.0	12.6	43.5	No symptoms
12/ 6/35	10:45 a.m.	108	80	140.6	113.0	11.9	37.9	No symptoms
D. Adrenalectomized. Dog 8♀—7.8 kgm. Maintenance hormone. No paracentesis								
11/22/35	11:15 a.m.	95	68	139.6	113.8	11.5	37.7	Normal. Glucose injected
11/22/35	2:35 p.m.	92	160	131.5	107.4	13.7	45.9	No symptoms
11/22/35	5:30 p.m.	80	160	131.2	106.2	15.2	48.5	Symptoms
11/22/35	8:30 p.m.	38	144	No samples taken				Collapse. Hormone injected
11/22/35	10:30 p.m.			132.3	105.8	15.0	48.2	Prostration
11/27/35	10:00 a.m.	92	88	138.2	115.8	9.3	30.8	Normal

0.2 to 0.5 cc. per kilogram daily. This dose was omitted the day of the experiment. A few hours after glucose injection, the animals were in collapse and verging on death and it was necessary to inject large doses of cortical hormone (3 cc. per kgm. daily) in order to save them. Despite the fact that the peritoneal cavity of these animals was filled with fluid, and their cells extensively hydrated, they were unable to draw upon or make use of any of these stores. This condition of affairs is just the reverse of that occurring when the same animals are primed previous to the glucose administration and subsequently injected with adequate cortical hormone. We have yet to observe a healthy, vigorous, adrenalectomized dog recover from the shock and collapse which follow the intraperitoneal injections of glucose, unless injected with either large doses of cortical hormone or sodium chloride. It does not matter whether the ascitic fluid in the depletion experiments is eventually removed by paracentesis or left within the peritoneal cavity of the adrenalectomized dog, since the animals are unable to utilize this fluid and electrolyte in the absence of hormone.

The healthy, vigorous adrenalectomized dog injected intraperitoneally with 50 cc. per kilogram of isotonic glucose and thereby thrown into shock and collapse, can be revived in dramatic fashion by intravenous injection of concentrated sodium chloride solution (30 per cent) containing an amount of salt equivalent to that removed by paracentesis in the ascitic fluid. The arterial pressure rises to normal from shock level (48 mm. Hg), within a few minutes. The animal rapidly recovers his strength and vigor and remains in normal condition for some time. During the ensuing 24 to 36 hours the blood pressure slowly falls. The recovery of these animals intravenously injected with salt is in striking contrast to the same type of animal prostrate and in collapse from exactly the same treatment but with the sodium and chloride in the ascitic fluid within the peritoneal cavity. The intravenously injected dog revives, whereas the animal with the same amount of salt in the ascitic fluid of the peritoneal cavity dies unless injected with cortical hormone.

The dog with intact adrenals suffers little ill effect from the glucose injections. The internal fluid balance is temporarily disturbed but the animal's own glands appear to bring about normal fluid distribution spontaneously. The majority of such animals do not exhibit symptoms following 50 cc. per kilogram injections. (Table 4C.)

Discussion. It is obvious from the experimental data that conditions in adrenal insufficiency are strikingly similar to those occurring in the healthy, vigorous, adrenalectomized animal depleted of sodium and chloride by intraperitoneal glucose. The two types of animal are physiologically comparable. They exhibit identical symptoms, similar changes in hemoconcentration, circulating fluid volume, arterial pressure and altera-

tions in the distribution of body water. Both lack ability to dilute the blood, i.e., redistribute the tissue fluids and both react in dramatic fashion to injections of cortical hormone.

In previous publications (Swingle, Pfiffner, Vars, Bott and Parkins, 1933, 1934a, b, c, d, e; Swingle and Parkins, 1935; Parkins, Taylor and Swingle, 1935) the writers have insisted that the dehydration and shock symptoms exhibited by the dog in adrenal insufficiency are chiefly due to a profound disturbance in the distribution of body water. The fluids shift from the blood stream into the tissues and are immobilized. The tissues imbibe unusual quantities of fluid at the expense of the circulating volume with consequent decrease in blood volume, arterial pressure, increased hemoconcentration and eventually complete circulatory collapse. This fluid loss can not be regained by the dog lacking adrenals and not supplied with hormone. Such animals have lost the ability to compensate by withdrawal of tissue fluid, and to dilute the inspissated blood and rebuild a normal circulating volume.

Loeb et al. (1933, 1935) and Harrop et al. (1933), maintain that the syndrome of adrenal insufficiency is due to wastage of fluid and salt to the exterior of the body by way of the urine. The kidney is assumed to be under hormonal control, and in the absence of the adrenal cortex loses its regulatory function in so far as the excretion of salt and water are concerned. These investigators would account in this way for the hemoconcentration and dehydration symptoms so characteristic of adrenal insufficiency. The writers agree that the animals do, to some extent, waste water and salt through the kidney, but maintain that the fluid loss in the urine is by no means of sufficient magnitude to induce the degree of dehydration and decrease in circulating volume which occurs. Fluid wastage by the kidneys does not occur in the "glucose" dog, and in our opinion, plays a minor rôle in the syndrome of adrenal insufficiency.

SUMMARY AND CONCLUSIONS

1. The intact dog depleted of sodium and chloride is sensitive to small blood loss subsequent to glucose injections and removal of ascitic fluid. Such animals recover without difficulty from hemorrhage of 10 cc. per kilogram. Restoration of the lost electrolytes is unnecessary for the revival of these animals.

2. The sodium and chloride-depleted intact dog is not strictly comparable to the healthy, vigorous, adrenalectomized animal subjected to similar depletion, or to the animal in adrenal insufficiency. The two latter types invariably succumb unless injected with adequate hormone since they lack adrenals, hence have permanently lost the ability to redistribute body fluid. The dog possessing glands suffers merely a temporary loss of diluting ability, and spontaneously recovers.

3. The adrenalectomized dog prostrate from insufficiency is the physiological counterpart of the adrenalectomized dog in collapse following intraperitoneal glucose injections. Both types of animal lack ability to dilute the blood, i.e., to release the excess fluids osmotically held within the tissue cells, and redistribute them to the extracellular spaces and blood, and both revive in dramatic fashion when injected with hormone.

4. The healthy, vigorous, adrenalectomized dog, if primed with large doses of cortical hormone previous to intraperitoneal glucose injection remains free from symptoms. The serum sodium and chloride decline to the levels characteristic of the non-primed animal in collapse. However, in the presence of adequate hormone the animals quickly dilute their blood and redistribute the body fluids in normal fashion.

5. The adrenalectomized dog in collapse as a result of sodium and chloride depletion by glucose injections, can be revived and restored to normal health and vigor temporarily, by a single *intravenous* injection of 30 per cent sodium chloride solution, the total amount of salt representing that quantity removed in the ascitic fluid by paracentesis. The same dog depleted of sodium and chloride in similar fashion, but with the ascitic fluid and its contained sodium and chloride left within the body cavity, dies from circulatory collapse unless injected with cortical hormone.

REFERENCES

Butler, A. M. and E. Tuthill. J. Biol. Chem. **93**: 171, 1931.

Darrow, D. C. and H. Yannet. J. Clin. Invest. **14**: 266, 1935.

Eisenman, A. J. J. Biol. Chem. **82**: 411, 1929.

Gilman, A. This Journal **108**: 662, 1934.

Harrop, G. A., A. Weinstein, L. J. Soffer and J. H. Trescher. J. Exper. Med. **58**: 1933.

Harrop, G. A., L. J. Soffer, R. Ellsworth and J. H. Trescher. J. Exper. Med. **58**: 17, 1933.

Loeb, R. F., D. W. Atchley, E. M. Benedict and H. Leland. J. Exper. Med. **57**: 775, 1933.

Loeb, R. F. J. A. M. A. **104**: 2177, 1935.

Parkins, W. M. This Journal **107**: 518, 1934.

Parkins, W. M., A. R. Taylor and W. W. Swingle. This Journal **112**: 581, 1935.

Silvette, H. and S. W. Britton. This Journal **111**: 305, 1935.

Swingle, W. W., J. J. Pfiffner, H. M. Vars, P. A. Bott and W. M. Parkins. Science **77**: 58, 1933.

Swingle, W. W., J. J. Pfiffner, H. M. Vars and W. M. Parkins. This Journal **107**: 259a; **108**: 144b; **108**: 159c; **108**: 428d; **109**: 488e, 1934.

Swingle, W. W. and W. M. Parkins. This Journal **111**: 426, 1935.

Van Slyke, D. D. and J. Sendroy, Jr. J. Biol. Chem. **58**: 523, 1923.

RELATION OF SERUM SODIUM AND CHLORIDE LEVELS TO ALTERATIONS OF BODY WATER IN THE INTACT AND ADRENALECTOMIZED DOG, AND THE INFLUENCE OF ADRENAL CORTICAL HORMONE UPON FLUID DISTRIBUTION

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In a recent paper (Parkins, Taylor and Swingle, 1935) brief mention was made of the fact that the adrenalectomized dog exhibiting severe dehydration and shock, either from adrenal insufficiency or as a result of intraperitoneal glucose injections, can be revived from prostration and restored to normal by hormone injections, with little or no change in the concentrations of serum sodium and chloride. It occurred to us that if the animals could be maintained for long periods with low levels of these electrolytes and observed through complete cycles of adrenal insufficiency and recovery, they would prove ideal material for study of the relation of the cortical hormone to the distribution of body water. It was with this object in view that the following experiments were undertaken.

MATERIAL AND METHODS. The adrenalectomized dogs employed were healthy, vigorous animals at peak weight. They had been bilaterally adrenalectomized six months previous, and maintained in normal health by adequate daily injections of cortical hormone. The intact animals were strong, active mongrels kept for six weeks in the laboratory before using so as to adjust them to the laboratory environment. The methods used have been repeatedly referred to in earlier papers of this series. In the sodium-chloride-depletion experiments on the intact dog, unless otherwise stated, the animals were injected intraperitoneally with 100 cc. of isotonic glucose per kilogram of body weight. After 3 to 5 hours, a paracentesis was performed and an amount of ascitic fluid equal to that injected was removed. This fluid was analyzed for sodium and chloride.

The diet of the animals maintained at low electrolyte levels, consisted of fresh, finely ground lean beef. The meat was soaked over night in a large volume of distilled water. This was decanted off and fresh distilled water added. The beef was thoroughly boiled, the broth poured off and the meat again boiled in fresh distilled water and this water discarded. By this procedure the beef is rendered practically free of sodium and chloride.

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A small amount of pure lard is added before feeding. The sodium-chloride-depleted dogs eagerly eat the food in large quantities. Despite the evident inadequacy of this diet from the standpoint of its nutrients and salts, the animals maintain themselves in excellent condition for periods in excess of three weeks. The experiments were discontinued at the end of this interval.

The animals were allowed free access to tap water. Prof. E. R. Caley of the Chemistry Department has for a number of years kept a record of the sodium and chloride content of Princeton tap water. The amounts

TABLE 1

Changes in serum sodium, chloride and hemoconcentration of an adrenalectomized dog observed through a cycle of insufficiency and recovery while maintained at low serum sodium and chloride levels

DATE	TIME	WEIGHT	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	Hb	CELL VOLUME	R.B.C.	UREA N	REMARKS
	hours	*kgm.*	*mm. Hg*		*m.-eq. per liter*	*m.-eq. per liter*	*grams per cent*	*per cent*	*millions*	*mgm. per cent*	
11/ 6/35	10:45 a.m.	7.6	102	68	140.8	114.2	11.5	38.2	5.29	33.3	Normal. Extract discontinued
11/12/35	10:30 a.m.	7.5	82	112							No symptoms
11/15/35	5:15 p.m.	7.4	65	72							Lethargic
11/16/35	4:15 a.m.	7.3	46	64	122.9	96.4	18.4	53.4	8.88	86.9	Severe insufficiency. Extract injected
11/17/35	9:45 a.m.	7.2	80	132							No food
11/20/35	10:30 a.m.	6.9	106	128	120.7	93.0	14.9	44.8	6.76	26.6	Active, vigorous. Salt free diet
11/23/35	10:25 a.m.	7.1	108	112	127.1	98.4	13.2	40.5	6.00	41.7	Extract discontinued
11/25/35	9:30 a.m.	7.1	98	140	126.3	97.6	13.6	43.1	6.58		"Saltfree" diet
11/29/35	10:45 a.m.	7.0	74	152							Listless
11/30/35	4:30 p.m.	6.8	48	132	127.5	97.6	16.0	47.8	7.63	181.8	Severe symptoms. Extract injected
12/ 1/35	3:30 p.m.	6.8	86	124			14.2	45.8	7.66	100.0	No food
12/ 2/35	10:00 a.m.	6.7	102	120	126.2	91.4	13.9	42.5	6.68	42.5	Appears normal
12/ 4/35	10:00 a.m.	6.6	103	132	126.4	91.8	12.7	43.0	6.61	25.9	"Saltfree" diet
12/ 6/35	10:10 a.m.	7.3	100	104	143.7	115.4	8.3	29.6	4.72	35.0	Salted diet

are: Na 0.82 m.-eq. and Cl 0.3 m.-eq. per liter. The concentration of these substances in the drinking water as given in the tables were calculated from Professor Caley's data.

I. *Experiments on the adrenalectomized dog observed through a cycle of insufficiency and recovery while maintained at low serum sodium and chloride levels.* Table 1 gives the pertinent data obtained from a representative experiment of this series of five animals.

The animal was in normal health on the day hormone injections were discontinued. Ten days later the dog was prostrate and on the verge of death from adrenal insufficiency. Cortical hormone was injected intrave-

nously in amounts representing 3 cc. per kilogram and thereafter this dose was given daily in two equally divided amounts, a.m. and p.m., until all symptoms had disappeared. Free access to water was permitted but all food withheld for 4 days subsequent to administering the first injection of hormone. The dog rapidly revived from insufficiency; activity, vigor and appetite returned. The arterial pressure rose from 46 mm. of Hg to 106 mm. Hg within 4 days. Hemoconcentration decreased as shown by decreases in hemoglobin, cell volume and erythrocyte count. The blood-urea nitrogen fell from 86.9 mgm. per cent to the normal level of 26.6 mgm. per cent. However, despite the obvious clinical recovery, the decreased hemoconcentration and evidence of blood dilution, the serum sodium and chloride remained unaltered at the levels observed when the dog was in collapse from insufficiency. In fact, as shown in table 1, this particular animal actually revealed a slight decline in the level of the serum sodium and chloride during the recovery interval. On 11/23/35 (table 1) the dog was in excellent condition and appeared normal except that the serum sodium and chloride were very low, although the levels of these electrolytes had risen somewhat indicating that slight amounts of sodium and chloride were present in the food. He was given the "salt free" diet and cortical hormone withdrawn. The animal remained free from symptoms for six days. On the 7th day the dog exhibited marked symptoms and was near collapse. He was injected with 3 cc. per kilogram of cortical hormone in divided doses. The animal promptly revived and all symptoms disappeared. The arterial pressure rose to the normal level, the hemoconcentration decreased and the blood-urea nitrogen was lowered from 181 mgm. per cent to 25.9 mgm. per cent. The animal was given plenty of water to drink but no food. *Throughout the entire cycle of insufficiency, prostration and recovery the serum sodium and chloride remained at extremely low levels.*

Experiments, identical in all respects to the one described, were performed upon four other adrenalectomized dogs. Two of the animals exhibited less striking alterations in hemoconcentration as evidenced by hemoglobin, cell volume and erythrocyte counts, than did the dog illustrated in table 1. However, all changes were well in the same direction as those discussed; revival from prostration and disappearance of all symptoms, etc., were identical. These dogs were maintained for approximately three weeks at low electrolyte levels. Throughout this interval the serum sodium was 115 m.-eq. and the serum chloride 93 m.-eq. in one of the dogs. At present we know of no good reason why these animals could not be maintained for longer intervals. Presumably the chief limiting factor would eventually be lack of proper nutrients.

B. *Sodium, chloride and fluid intake and output during a cycle of insufficiency and recovery of an adrenalectomized dog maintained at low serum sodium and chloride levels.* In table 2 are shown typical data obtained

from a representative case in this series of experiments performed on four adrenalectomized animals. These data were collected on the dog discussed in table 1. The fluid balances are incomplete to the extent that metabolic water was not calculated, and the water lost by evaporation, in the expired air and feces was not determined. Diarrhea and vomiting did not occur.

The treatment the animal received immediately prior to collection of data on fluid balance, may be seen by study of table 1. Hormone was discontinued and the dog permitted to develop severe insufficiency. He

TABLE 2

Sodium, chloride and fluid intake and output during a cycle of insufficiency and recovery of an adrenalectomized dog maintained at low serum sodium and chloride levels.
(*Daily averages*)

EXPERIMENTAL PERIOD	WATER DRUNK	WATER* INTAKE	URINE VOLUME	SODIUM† INTAKE	SODIUM OUTPUT	CHLORIDE† INTAKE	CHLORIDE OUTPUT	REMARKS
days	*cc.*	*total cc.*	*cc.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	
Insufficiency 11/23 to 11/30/35	307	490	257	0.25	0.90	0.092	1.95	No hormone. "Salt free" diet
Recovery 1 11/30 to 12/4/35	328	352	258	3.63	1.34	3.46	3.02	Hormone. 3 cc./kgm. No food
Recovery 2 12/4 to 12/6/35	800	972	558	137.1	72.8	136.7	79.3	Hormone. 1.5 cc./kgm. Salted diet
Normal 13 12/6 to 12/9/35	783	970	644	155.5	138.6	156.7	144.0	Maintenance hormone. Salted diet

* Includes water in the food and extract.

† Calculated from the Na and Cl content of water drunk, NaCl in the isotonic extract and the NaCl added to the "salt free" food.

was then revived from prostration and restored to normal vigor by adequate doses of hormone. During the first recovery phase, water was allowed *ad lib.*, but not food. The first data on water balance were collected on 11/23/35. On this date the dog was receiving the "salt free" diet and had low serum electrolytes but was otherwise in normal condition.

Table 2 summarizes the data. Comparison of this table with table 1 reveals at once that during the insufficiency phase of the cycle, the dog maintained at low serum sodium and chloride levels on the "salt free" diet loses considerably *less* fluid (in relation to total fluid intake) by way of the urine than he does during the recovery phase on extract treatment.

The average daily total water intake of the dog developing insufficiency is more than the average daily total intake during recovery. The dog

received no food in the recovery period, whereas, following extract withdrawal, he continued to eat the "salt free" diet and consequently ingested more fluid. Although the figures in table 2 show that the average daily urine volume was practically identical during both insufficiency and recovery phases, actually, however, the animal lost more fluid in relation to intake during recovery than he did in insufficiency since the total fluid intake was less in the latter phase of the cycle.

The animal was fed the "salt free" diet after complete disappearance of all symptoms following extract treatment, and given 3 grams of sodium chloride per 100 grams of food. The total quantity of salt was 9 grams per day (recovery period 2, table 2). During this salt feeding period the animal ingested large quantities of water, urinated freely and retained sodium and chloride. When at the end of two days on salt, the animal had restored the serum electrolytes to their usual concentrations, the sodium and chloride intake and output became fairly evenly balanced. This is shown in normal period 3 as compared with recovery period 2, table 2. The daily hormone requirements dropped to a small fraction of that necessary to maintain normal vigor and activity at low electrolyte levels on a salt free diet.

II. *Depletion of sodium and chloride in the intact dog by intraperitoneal injections of isotonic glucose, and maintenance of the animal at low serum electrolyte levels.* In these experiments, two successive sodium and chloride "depletions" were carried out on the same dog. The animal was injected intraperitoneally with 100 cc. of isotonic glucose per kilogram and the ascitic fluid withdrawn at the end of three hours, care being taken to remove only the same amount of fluid as was injected. Immediately a second injection of 100 cc. per kilogram of glucose was given. The peritoneal fluid was withdrawn after 2 hours. By this method it is possible to reduce the serum sodium and chloride to extremely low levels. The dogs develop severe symptoms of dehydration and shock, with drastic reduction in blood volume, arterial pressure, increase in hemoconcentration and urea nitrogen. However, the animals recover spontaneously after 18 to 36 hours, and can be maintained in apparently normal health and vigor for long periods at low serum electrolyte levels if given the "salt free" diet.

The essential data obtained from study of a representative case are given in table 3. It will be observed that the animals redistribute their body fluids in practically normal fashion at low serum electrolyte levels. The arterial pressure rises to normal, the hemoconcentration decreases and all symptoms disappear. Despite the low levels of serum sodium and chloride the dogs dilute their blood by withdrawal of tissue fluid. It is obvious that the greatest dilution changes occur during the first 72 hours following electrolyte depletion. Following this interval dilution slows

down and the hemoglobin, cell volume, R.B.C. and blood-urea nitrogen tend to remain stabilized at levels above the normal. The arterial pressure, however, invariably returns to normal. If the animals are injected with cortical hormone, 2 cc. per kilogram per day in divided doses for two days the serum electrolytes reveal negligible change but the levels of the hemoglobin and blood-urea nitrogen are reduced. The urea-nitrogen tends to rise again when the injections are stopped but the hemoglobin continues to fall until it reaches a level below normal. Following the addition of

TABLE 3

Blood changes following sodium and chloride depletion in the intact dog by intraperitoneal injections of isotonic glucose, and maintenance of the animal at low serum electrolyte levels

DATE	TIME	WEIGHT	BLOOD PRESSURE	PULSE PER MINUTE	SERUM SODIUM	SERUM CHLORIDE	Hb	CELL VOLUME	R.B.C.	UREA N	REMARKS
	hours	*kgm.*	*mm. Hg*		*m.-eq. per liter*	*m.-eq. per liter*	*grams per cent*	*per cent*	*millions*	*mgm. per cent*	
2/15/36	7:30 a.m.	16.2	106	92	143.0	114.0	14.9	42.1	7.44	18.6	Glucose injected*
2/15/36	12:45 p.m.		62	164	114.4	83.8	20.9	67.5	11.43	21.2	Paracentesis.† Weak
2/17/36	10:00 a.m.		100	140			18.7	57.5	9.28	86.9	Strong, active
2/18/36	10:30 a.m.	14.7	110	144	112.8	76.8	17.5	56.5	9.11	62.4	"Saltfree" diet
2/19/36	10:30 a.m.	14.4	108	140			17.9	55.2	9.30	50.0	"Saltfree" diet
2/20/36	10:20 a.m.	14.2	110	152			17.3	52.3	8.76	42.7	"Saltfree" diet
2/21/36	10:15 a.m.	14.2	112	136	111.4	77.4	17.6	54.1	9.21	46.5	Extract 1 cc./kgm.
2/21/36	10:45 p.m.		108	132			16.2	48.3	8.64		Extract 1 cc./kgm.
2/22/36	9:50 a.m.	14.1	100	116	113.4	78.2	16.2	49.7	8.62	30.3	Extract 1 cc./kgm.
2/23/36	10:30 a.m.	13.9	108	136	112.5	78.2	15.1	49.1	8.21	30.7	Active, vigorous
2/24/36	10:00 a.m.	13.8	106	140			15.7	50.8	8.49	41.7	"Saltfree" diet
2/26/36	10:00 a.m.	13.6	108	140	118.2	81.8	14.6	46.7	8.01	40.8	"Saltfree" diet
2/29/36	10:00 a.m.	13.6	110	132	119.8	83.2	13.5	44.3	7.78	40.8	"Saltfree" diet
3/ 1/36	10:00 a.m.	14.0	112	124	139.7	110.8	12.4	39.9	6.61	18.6	Salted diet
3/ 2/36	10:15 a.m.	14.0	108	88	149.0	120.4	13.1	42.7	6.65	20.0	Experiment discontinued

* 100 cc./kgm. Paracentesis 3 hours later. 2nd injection 100 cc./kgm. Paracentesis 2 hours later.
† Total of 261 m.-eq. sodium and 226 m.-eq. chloride removed in 3290 cc. of ascitic fluid.

salt to the diet the serum sodium and chloride are rapidly elevated to normal and the blood-urea nitrogen falls.

It is evident that the adrenal glands of the sodium-chloride-depleted dog supply sufficient hormone to induce marked decrease in hemoconcentration and disappearance of all dehydration symptoms. However, the hormone supply is inadequate to restore completely the normal fluid balance in the face of such low electrolyte levels unless augmented by hormone injections.

The observation that injections of the cortical hormone by reinforcing the action of the animal's own glands reduced the hemoconcentration of the salt-depleted intact dog to normal levels is interesting in view of the fact that this is the first time the writers have been able to induce a physi-

ological response of any kind in the unoperated animal with cortical hormone. It is probable that these observations can be made the basis of a bio-assay of cortical hormone based on the unoperated salt-depleted dog. Experiments along this line are now under way in this laboratory.

Discussion. These experiments demonstrate that the symptoms and clinical condition of the adrenalectomized dog exhibiting insufficiency are independent of the serum sodium and chloride levels when adequate cortical hormone is administered. The writers can only hazard a surmise as to the probable nature of the mechanism involved in the redistribution of the body fluids under influence of adrenal cortical hormone when the serum sodium and chloride are at low levels and remain so throughout the cycle of adrenal insufficiency and recovery on extract. It is evident that the phenomenon is an osmotic one, but it is far from clear as to how the hormone can restore the altered water distribution to approximately normal condition in the face of such marked disturbances in osmotic relationships as must obtain when the serum sodium and chloride levels are so greatly reduced. However, there are several possibilities. The hormone apparently does not change the sodium and chloride levels when injected in large quantities but does redistribute the body fluids. 1. Does it induce this internal fluid shift by directly restoring osmotic equilibrium, i.e., is the cortical hormone *per se* a highly active osmotic agent in the sense that the electrolytes are such agents? This seems improbable in view of the marked lag period between injection and response to this hormone. 2. Does the hormone, when present in adequate amounts, "activate" the sodium and chloride remaining in the body fluids of the adrenal insufficient or salt depleted dog, so that 80 m.-eq./1. of serum chloride and 114 m.-eq./1. of serum sodium, in the presence of quantities of hormone or hyperactive glands are osmotically equivalent to 110 m.-eq./1. of chloride and 143 m.-eq./1. of sodium in the fluids of the normal animal? 3. As a third possibility it may be that the cortical hormone when given in sufficient quantity to animals with low serum sodium and chloride levels so alters the permeability of the cell membranes that osmotically active substances normally retained within the cells pass outward through the restraining envelope thereby raising the osmotic pressure of the hypotonic extracellular environment and redistributing the fluid osmotically held within the cells.[2]

[2] The statement has been attributed to the writers that the permeability of the capillaries is altered following adrenalectomy. We do not believe, nor have we ever implied in any publication, that the permeability of the capillaries is significantly changed from the normal in the adrenalectomized dog. On the contrary we have expressed the belief that the cortical hormone is probably not concerned with capillary permeability. (Swingle, Pfiffner, Vars and Parkins. This Journal **107**: 271, 1934.)

SUMMARY AND CONCLUSIONS

Five adrenalectomized dogs were studied through a cycle of insufficiency and recovery while maintained at low serum sodium and chloride levels. 1. The clinical condition of the animals so treated is independent of the serum sodium and chloride levels when adequate amounts of cortical hormone are administered. 2. The animals can be maintained apparently normal in so far as health and vigor are concerned for three weeks (and probably longer) with the serum sodium and chloride at the levels observed when the animals are verging on death from insufficiency. 3. The disappearance of symptoms is largely due to the effect of the cortical hormone on the mobilization and shift of tissue fluids to the extracellular spaces and blood stream. This shift occurs despite the low serum sodium and chloride concentration. 4. The serum sodium and chloride concentrations remain unaffected by repeated large injections of cortical hormone if all food is withheld or a salt-free diet fed. 5. Water balance studies on four animals show that the animals coming into adrenal insufficiency while maintained at low serum sodium and chloride levels lose less fluids by way of the urine than they do during the recovery phase while receiving extract. 6. The urinary excretion of sodium and chloride in the salt-depleted dogs during insufficiency is very small.

7. Intact dogs, depleted of sodium and chloride by intraperitoneal injections of isotonic glucose, can be maintained healthy and vigorous for three weeks with extremely low levels of serum sodium and chloride. 8. Sufficient cortical hormone is elaborated by the animal's own adrenals to restore the altered fluid distribution to approximately normal condition without change in the level of the serum sodium and chloride. 9. Injections of hormone, supplementary to that secreted by the animal's own glands, rapidly reduces the hemoconcentration and blood urea nitrogen to normal. 10. Possible modes of action of the hormone are discussed.

REFERENCES

HARROP, G. A., L. J. SOFFER, R. ELLSWORTH AND J. H. TRESCHER. J. Exper. Med. **58**: 17, 1933.

HARROP, G. A., L. J. SOFFER, W. M. NICHOLSON AND M. STRAUSS, JR. J. Exper. Med. **61**: 839, 1935.

LOEB, R. F., D. W. ATCHLEY, E. M. BENEDICT AND J. LELAND. J. Exper. Med. **57**: 775, 1933.

LOEB, R. F. J. A. M. A. **104**: 2177, 1935.

PARKINS, W. M., A. R. TAYLOR AND W. W. SWINGLE. This Journal **112**: 581, 1935.

SWINGLE, W. W., J. J. PFIFFNER, H. M. VARS AND W. M. PARKINS. This Journal **108**: 144, 1934.

This Journal **108**: 159, 1934.

SWINGLE, W. W., W. M. PARKINS AND A. R. TAYLOR. Proc. Soc. Exper. Biol. and Med. **34**: 75, 1936.

THE EFFECT OF DIETS AND VARIOUS SUBSTANCES ON THE VITAMIN C CONTENT OF SOME ORGANS OF THE RAT

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Biologic tests have indicated that the rat is able to synthesize its own vitamin C. Varying concentrations of ascorbic acid (vitamin C) have been found in the organs of the rat by direct titration with the indophenol indicator (1, 2). The results of the titration method, extensively used at the present time, agree closely with those of biological tests. The distribution of ascorbic acid in the organs of the body, especially the high amounts in the small intestine of those animals which are not susceptible to scurvy, has been adequately reviewed (3).

Evidence has been presented that, in the germinating pea seedling (4), as well as experiments *in vitro* with certain tissues of the rat (5), mannose alone of the many sugars tested seemed to serve as the most effective precursor of vitamin C. Additional work, however, has not indicated that mannose could be converted into significant amounts of ascorbic acid either *in vitro* (6), or *in vivo* (7).

A review of the literature suggested a careful investigation of the distribution of vitamin C in the organs of the rat when these animals were fed various diets for definite periods of time in order to obtain further information as to the probable site of synthesis of ascorbic acid as well as to the nature of its precursors.

EXPERIMENTAL. Young albino rats, reared in this laboratory from a closely inbred strain, were used in these experiments. Animals from such a strain are less apt to show extreme variations as regards growth and the vitamin C content of their organs. Groups of three to five rats were placed in cages with raised screen grids (one-half inch mesh). Liberal quantities of the diets under consideration (estimated at about 10 grams daily to each rat) were given to the animals which had shown normal growth on the basal diet of dog chow. Water was kept before the animals at all times. Weekly records of the body weight as well as the age of the animals were kept.

At definite time intervals, generally 16 to 18 hours after the last feeding, the rats were killed by stunning. The organs were removed (the

small intestine being gently "milked" with the fingers to expel the contents), dried between sheets of filter paper, weighed, and minced finely with scissors. The minced organs were extracted with a sufficient quantity of 4 per cent trichloroacetic acid for at least five minutes. The filtered solution (the residue being washed with additional acid) or an aliquot portion was then titrated rapidly to the first permanent trace of pink color with standardized 2,6 dibromophenol indophenolblue. Extreme variations in the weight of the organs or titration values among the individual rats of each group were seldom observed. Fluctuations in the weight of

TABLE 1

Components and percentage composition of various diets used in this investigation

COMPONENTS	VITAMIN B COMPLEX DEFICIENT	VITAMIN B_1 DEFICIENT	VITAMIN B_2 DEFICIENT	CASEIN FREE*	HIGH STARCH	CARBOHYDRATE FREE	LACTOSE	CONTROLS
Extracted casein	18		15					
Technical casein		16			15	55		
Ground whole wheat			20					
Osborne and Mendel salts	4	4	4	4	4			
Cornstarch	30	30	26	34	61			
Sucrose	29	30	25	33				
Cod liver oil	2	2	2		2			
Lard				17		15		
Crisco	9				9			
Butterfat	8	9	8	7		30		
Dried yeast		9†		8	9	‡		
Lactose (C.P.)							33	
Purina Dog Chow							66	ad lib.

* Rats refused to eat the casein free diet readily until yeast was substituted for Vitavose as source of vitamin B factors and nitrogen.

† Dried yeast autoclaved for 5 hours at 15 pounds' pressure.

‡ Five-tenths gram dried yeast given daily to each rat.

the spleen were occasionally recorded. The average weight of the organs can be easily ascertained from the data in the tables since both the total ascorbic acid content as well as the amount per gram of tissue have been calculated.

The composition of the diets used in this investigation is given in table 1.

Rats, fed exclusively on a dog chow ration (Ralston Purina Dog Chow), were chosen as control animals. This basal ration contains all the necessary ingredients for normal growth and the rats ate it readily. Some of the other diets were deficient in a dietary factor as vitamin B, protein (casein-free), or carbohydrate but were adequate in all other constituents.

Rats fed on the "high starch diet," which to be true did not contain more carbohydrate than the other starch diets, actually consumed more carbohydrate daily since this was the only starch diet which contained all the necessary ingredients for normal growth. Substitution of yeast for vitavose in the casein-free diet resulted in a more improved appetite of the rats. A slight gain in weight in 18 days (see table 3), was then recorded which, however, was still below the initial weight of the animals before being placed on this diet.

TABLE 2

Vitamin C content of organs of rats on vitamin B deficient diets

DIET FED	NUMBER AND SEX OF ANIMALS	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	AVERAGE GAIN IN WEIGHT	TEST PERIOD	FINAL AGE WHEN KILLED	VITAMIN C CONTENT OF ORGANS					
							Adrenals	Liver	Spleen	Adrenals	Liver	Spleen
		gms.	*gms.*	*gms.*	*days*	*days*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per grm.*	*mgm. per grm.*	*mgm. per grm.*
Vitamin B complex deficient	5 M	49	39	−10	29	55	0.033	0.192	0.024	1.86	0.12	0.17
	3 F	40	31	−9	29	55	0.022	0.115	0.016	1.93	0.10	0.39
Vitamin B_1 deficient	5 F	40	35	−5	29	55	0.024	0.124	0.012	2.44	0.08	0.18
	2 M	37	66	29	33†	59	0.041	0.940	0.047	3.22	0.18	0.15
Vitamin B_2 deficient*	6 F	44	64	20	32	58	0.045	0.369	0.045	2.75	0.16	0.16
	3 M	50	67	17	38	64	0.030	0.382	0.038	2.47	0.13	0.15
	3 M	47	61	14	38‡	64	0.037	0.740	0.054	2.56	0.19	0.14
Dog chow controls	3 M	42	151	109	29	55	0.047	1.712	0.137	2.26	0.24	0.20
	3 F	46	131	85	29	55	0.093	1.035	0.133	3.11	0.18	0.16
	3 M	44	196	152	43	70	0.060	2.157	0.180	2.38	0.24	0.19
	2 F	50	159	109	39	65	0.087	1.072	0.134	2.56	0.16	0.15

* Contains slight amounts of vitamin B_2 from ground whole wheat.

† Dried yeast given last 7 days of test.

‡ Dried yeast given last 4 days of test.

For uniformity of results, it is essential that the animals eat the diet under consideration to as great extent as possible. Otherwise, inanition over a great period of time may give subnormal values for the vitamin C content of the organs. Furthermore, the test period must be of sufficiently long duration to have less fluctuation in the normal metabolism as the result of the shifting from the basal to the experimental diets. The age, weight, and sex of the animals should be indicated as these factors are essential for uniform interpretation of the experimental data.

Influence of diets: Vitamin B deficient diets. As evident from table 2,

rats maintained on diets wholly free from vitamin B complex, vitamin B_1 and partially deficient vitamin B_2 are still able to synthesize their own vitamin C to a certain extent though showing many of the symptons characteristic of these deficiencies. The ascorbic acid content of the adrenals and liver was significantly lower in those diets entirely devoid of the vitamin B factor studied. These lower values of ascorbic acid in the organs may be the result of inanition and disarrangement of the metabolic processes but these symptons are intimately associated with such deficiency diseases. Adequate amounts of vitamin B factors are essential for the normal synthesis of vitamin C since the addition of yeast in a relatively short period not only caused resumption of growth but also raised the vitamin C content of the organs.

Other diets. Of the organs which can possibly take part in the synthesis of ascorbic acid in those animals, immune to scurvy, only the liver and gut (small intestine) need be considered. Adrenalectomized dogs are capable of synthesizing their own vitamin C (8). The slight amounts actually present in the pituitary, adrenals, spleen, or the other organs, precludes them from any serious consideration as to the possible sites of synthesis of this vitamin. Such organs can be removed from dogs, rats, or mice without serious departures from the normal condition under suitable experimental precautions.

It is apparent from table 3 that rats are able to synthesize vitamin C irrespective of the composition of the diet. The data indicate that the ascorbic acid content of the gut tends to be significantly higher than that of the liver on all the diets except the casein-free diet where no marked change occurs. Since the vitamin C content of the organs was made on the same animal, these rats are standardized insofar as other sources of disturbance such as age, weight, and metabolic condition are concerned. The relative difference in concentration of ascorbic acid in the liver and gut, especially of the rats fed the carbohydrate-free, lactose and control diets, as well as the fasting male rats, strongly point to the assumption that the small intestine is the source yielding primary precursors for the synthesis of vitamin C. The rate of absorption of the end products from the intestine into the liver varies with the nature of the foodstuff undergoing digestion and would be more rapid from carbohydrates than fats or proteins. In such a case, the amount of ascorbic acid in the liver may be variable depending on the rate of absorption of the metabolic products from the gut. Attention already has been drawn to the fact that the experiments *in vitro* (5), tending to indicate that the liver plays a limited rôle in the synthesis of ascorbic acid from mannose alone of the many sugars tested, have not been confirmed (6). The other organs of the digestive system play a minor rôle in the digestion of foodstuffs and the vitamin C content

of the cecum and large intestine may result from absorption of the metabolic products from the gut.

The apparent utilization of lactose by the rat (a slight tendency toward diarrhea was noted on this diet) is of interest on account of the synthesis of ascorbic acid from galactose (9). It has been definitely proven that the gut, as well as the liver, is able to utilize fructose and that appreciable amounts of galactose are metabolized even in the absence of the liver (10).

TABLE 3

Vitamin C content of organs of the rat on different diets

DIET FED	NUMBER AND SEX OF ANIMAL	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	AVERAGE GAIN IN WEIGHT	TEST PERIOD	AGE WHEN KILLED	VITAMIN C CONTENT OF ORGANS							
							Adrenals	Liver	Small intestine	Spleen	Adrenals	Liver	Small intestine	Spleen
		gms.	*gms.*	*gms.*	*days*	*days*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm.*
Casein-free	5 F	55*	63	8	18	72	0.037	0.444	0.490	0.026	2.62	0.17	0.16	0.16
High starch	3 F	111	156	45	22	73	0.076	0.775	0.725	0.066	2.10	0.11	0.15	0.14
Carbohydrate-free	4 F	127	171	49	22	73	0.106	0.927	0.970	0.065	2.60	0.12	0.17	0.17
Lactose (33 per cent)	5 F	84	108	24	32	74	0.061	0.754	1.474	0.082	3.31	0.21	0.29	0.16
	3 F†	93	104	11	15	61	0.076	0.527	1.484	0.051	3.45	0.14	0.28	0.24
Controls, dog chow	4 F		40			25	0.020	0.400	0.728	0.024	2.91	0.25	0.37	0.18
	3 F‡		91			49	0.049	0.793	1.094	0.057	3.31	0.19	0.26	0.23
	2 F	115	150	35	14	68	0.110	0.955	1.270	0.105	3.39	0.14	0.22	0.13
	1 F		208				0.128	0.992	1.540	0.088	4.00	0.11	0.23	0.09
	4 M		60			26	0.023	0.808	1.016	0.038	2.94	0.28	0.32	0.19
	2 M	145	165	20	4	67	0.068	1.723	1.841	0.121	3.04	0.24	0.31	0.12
	3 M§		353			270	0.070	2.51	1.720	0.148	2.74	0.21	0.25	0.20
	3 M¶		356			270	0.087	2.00	1.77	0.142	3.08	0.13	0.25	0.20
(Fasted)	3 M	181	135	−46	4	72	0.102	0.836	1.035	0.065	3.43	0.17	0.26	0.11

* Initial weight taken when yeast substituted for vitavose. Had lost 21 gms. in weight in previous 14 days.

† Vitamin C content of other parts of the digestive system: Stomach, 0.08 mgm. per gram; cecum, 0.12 mgm. per gram; large intestine, 0.14 mgm. per gram.

‡ Vitamin C content of other parts of the digestive system: Stomach, 0.10 mgm. per gram; cecum, 0.16 mgm. per gram; large intestine, 0.17 mgm. per gram.

§ Vitamin C content of other parts of the digestive system: Stomach, 0.07 mgm. per gram; cecum, 0.11 mgm. per gram; large intestine, 0.10 mgm. per gram.

¶ Etherized.

Data published from another laboratory (11) also point to a wide variation in vitamin C content of the gut and liver depending on the type of incomplete diet (i.e., incomplete in the sense that one or more of the three main organic foodstuffs was lacking) fed to male rats. A marked increase in the ascorbic acid content of the gut was present on the protein-fat and fat diet as compared to the liver whereas on a carbohydrate diet alone, the opposite was found to be the case. Such marked concentrations

were available only in a test period of 5 to 7 days, for on longer periods of 10 to 12 days, the absolute concentrations were much lower in both organs.

It is further evident from the table that ascorbic acid per gram of gut and liver tends to be higher in younger than older animals, confirming previous work (2). The opposite was generally found to occur in the case of the adrenals, indicating that the localized concentration of vitamin C is concerned with the maintenance of the normal functional activities of that organ (12).

A slight decrease of ascorbic acid was noted in the liver when the rats were etherized instead of killed by stunning. No adequate explanation is available why ether should give the lower values in the case of the liver.

TABLE 4

Comparison of the relative weights of the liver and small intestine

DIET	FINAL WEIGHT OF FEMALE RATS	WEIGHT OF LIVER	WEIGHT OF GUT	WEIGHT OF LIVER	WEIGHT OF GUT
	gms.	*gms.*	*gms.*	*gms. per 100 gms. final body weight*	
Casein-free	63	2.55	3.18	4.04	5.04
High starch	156	6.37	5.03	4.09	3.22
Carbohydrate-free	171	7.67	5.69	4.64	3.33
Lactose	108	3.61	5.00	3.34	4.62
	104	3.64	5.30	3.50	5.09
Dog chow	40	1.56	2.05	3.90	5.10
	91	3.97	4.09	4.40	4.50
	150	6.94	5.78	4.62	3.86
	208	8.62	6.60	4.14	3.17
Phloridzinized rats	117	6.22	8.82	5.31	7.54
Phloridzinized rats (table 5)	94	4.76	5.67	5.06	6.07

A greater elimination of ascorbic acid in the urine of guinea pigs has been recorded when ether had been used as the general anesthetic (13).

Comparison of the relative weights of the liver and gut. A possible explanation for the variation of the ascorbic acid content in the organs as regards age and diet fed to the rats is indicated in table 4. Other factors may exert their influence but it is significant that the ascorbic acid content of the organs is correlated with the weight of the small intestine and the liver. From the data in the previous table, it is apparent that a mechanism of some kind must be present through which a mutual adjustment in the productive activities of these organs can be established.

Influence of various substances on the vitamin C content of the organs of the rat. The addition of cincophen at levels where no difficulty was encountered on refusal of the animals to eat the basal diet resulted in a de-

crease in the ascorbic acid content of the liver. No macroscopic evidence of liver damage was noted.

TABLE 5

Influence of various substances on the ascorbic acid content of the organs of the rat

FED DAILY, THE FOLLOWING SUBSTANCES INTIMATELY MIXED WITH DOG CHOW	NUMBER AND SEX OF ANIMALS	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	AVERAGE GAIN IN WEIGHT	TEST PERIOD	AGE WHEN KILLED	VITAMIN C CONTENT OF THE ORGANS							
							Adrenals	Liver	Small intestine	Spleen	Adrenals	Liver	Small intestine	Spleen
mgm. approximately		*gms.*	*gms.*	*gms.*	*days*	*days*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm.*	*mgm per gm.*
Cincophen														
50	5 M	161	251	54	56	111	0.089	1.200	1.576	0.104	3.63	0.14	0.25	0.17
75	5 M	154	185	31	56	111	0.068	1.116	1.844	0.109	3.53	0.15	0.28	0.17
α-dinitrophenol														
10	3 F	134	140	6	25	108	0.044	0.671	1.120	0.096	1.93	0.11	0.22	0.11
10	3 F	96	109	13	42	97	0.049	0.465	0.707	0.050	2.37	0.09	0.15	0.09
20	5 F	97	97	0	37	88	0.046	0.497	0.736	0.064	2.28	0.09	0.15	0.11
Desiccated thyroid														
30	5 F	90	96	6	42	94	0.053	0.468	0.854	0.045	1.94	0.11	0.17	0.14
30	4 M	87	109	22	32	74	0.068	0.738	0.885	0.065	2.75	0.13	0.18	0.14
60	2 M	110	96	−14	22	70	0.062	0.625	0.562	0.040	2.40	0.14	0.15	0.08
125	3 M	134	126	−8	8	61	0.039	0.735	1.015	0.043	1.70	0.11	0.15	0.11
125	4 M	147	125	−22	12	65	0.066	0.868	0.951	0.083	2.49	0.14	0.18	0.14
Thyroid plus salts*														
125	3 M	149	119	−30	8	61	0.047	0.630	1.245	0.038	1.63	0.13	0.16	0.11
125	4 M†	155	112	−43	11	64	0.048	0.304	0.640	0.027	1.47	0.05	0.12	0.06
NaF (15 mgm.)	4 M	94	125	31	33	75	0.070	1.160	1.620	0.088	3.44	0.23	0.29	0.15
NaF (15 mgm.) plus thyroid (30 mgm.)	4 M	212	186	−26	19	85	0.076	0.970	1.020	0.085	2.20	0.12	0.15	0.11
Phloridzin (50 mgm. per ml. olive oil) injected subcutaneously	2 M‡	77	113	36	12	50	0.093	1.23	2.71	0.116	3.47	0.20	0.32	0.22
	3 F‡	89	117	28	12	50	0.096	0.970	2.37	0.10	3.16	0.15	0.27	0.22
	3 F§	76	94	18	5	49	0.064	0.817	1.371	0.054	3.76	0.17	0.24	0.22

* Salts consisted of a mixture of sodium citrate and sodium chloride (1:2). Each rat ate about 0.8–1 gram daily. A 0.9 per cent salt solution was given in place of ordinary water for drinking.

† Rats in state of collapse and dying. Organs extracted within one-half hour after death.

‡ Rats killed 24 hours after last injection. Total of 5 injections (on alternate days).

Vitamin C content of other organs of the digestive system (mgm. per gm.):

Stomach: Males, 0.08; females, 0.06.

Cecum: Males, 0.15; females, 0.12.

Large intestine: Males, 0.12; females, 0.07.

§ Killed 48 hours after last injection—2 injections only on alternate days.

The data in table 5 conformed with experiments on guinea pigs (14), that the ascorbic acid content of the organs tends to be decreased as the metabolism of the animal is increased under the influence of α-dinitrophenol or desiccated thyroid gland. Normal male rats, 135 to 270 grams in weight, have been shown (11) to contain 0.26 mgm. ascorbic acid per

gram of gut and liver respectively. These values agree well with those obtained in this laboratory.

The addition of sodium salts which have a noticeable influence in keeping adrenalectomized dogs in normal condition (15) have no effect when given in conjunction with the high level of thyroid gland fed. Incubation of minced rat liver of thyroid-fed rats (125 mgm. level) for 70 minutes at room temperature (27°C.) showed no decrease in ascorbic acid value. Consequently, the extremely low values for the vitamin C content of the organs of the rat at the point of collapse as the result of excess thyroid feeding must be ascribed to a more complete utilization of ascorbic acid as the metabolic condition of the animal is increased.

Previous work that sodium fluoride does not affect the ascorbic acid content of the adrenals and liver (16) is confirmed. The normal values for the vitamin C content of the gut is of interest in view of the reported similarity between scurvy and fluorosis in the guinea pig (17). The symptoms, hypertrophy of the adrenals, loss in weight and intestinal hemorrhages, listed in the fluorosis of guinea pigs may be obtained with other substances (administration of carbon tetrachloride to rats) and the intermuscular hemorrhages, characteristic in scurvy, were seldom observed. The experimental data (17) indicate that a decrease in the ascorbic acid content of the adrenals and pituitary does occur in fluoride-fed guinea pigs. This does not mean, however, that fluoride feeding should result in a marked interference with the action of vitamin C in the guinea pig especially when there was no decrease in the ascorbic acid content of the liver.

The combination of sodium fluoride and desiccated thyroid, at a level where either substance alone has no appreciable effect on exhaustion of body weight or metabolic condition (18), markedly decreases the ascorbic acid content of the organs.

Rats subjected to injections of phloridzin in olive oil showed the characteristic swelling of the liver and a marked increase in weight as well as thickening of the intestinal mucosa which was dependent on the degree of phloridzinization. Such a marked increase in weight was not observed in the stomach, cecum or large intestine. The response to phloridzinization, in view of the similarity of the relative concentration of ascorbic acid in the organs as compared to that of the controls and also the well-known physiologic action of this drug, offers additional proof to the ready adaptability of the gut to cope with experimental conditions which are not normal in the life of the animal.

Effect of carbon tetrachloride. Male rats maintained for two weeks on the diets indicated in the table were subjected to carbon tetrachloride vapor for a period of 30 to 45 minutes daily, six days a week. The survival time and general vitality of the rats on this treatment depended, as

previously reported (19), on the nature of the diet—the most severe reaction being produced on the carbohydrate-free diet. Owing to the brief experimental period in subjecting the rats to carbon tetrachloride, marked cirrhotic livers were not observed but the livers were definitely hard and fatty in all the diets fed. The increase in weight of the adrenals,[1] the presence of intestinal hemorrhages on the dog chow but not on the starch diet, and the loss in weight of the rats depended on the reaction of the animal to carbon tetrachloride. The data in table 6 indicate that even with fatty livers and possible disarrangement of hepatic function, appreciable amounts of vitamin C are synthesized by the rat. The relative

TABLE 6

Effect of carbon tetrachloride vapor on male rats fed various diets

DIET	NUMBER OF RATS	INITIAL WEIGHT	FINAL WEIGHT	GAIN IN WEIGHT	TREATMENTS OF CCl_4	TEST PERIOD	FINAL AGE WHEN KILLED	VITAMIN C OF CONTENT ORGANS*							
								Adrenals	Liver	Small intestine	Spleen	Adrenals	Liver	Small intestine	Spleen
		gms.	*gms.*	*gms.*		*days*	*days*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per organ*	*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm.*	*mgm. per gm.*
Dog chow	5†	138	107	−31	11	12	62	0.099	0.542	0.506	0.082	2.64	0.10	0.11	0.12
	7	137	155	18	21	25	74	0.145	1.718	1.442	0.207	3.32	0.15	0.21	0.17
	3	150	151	1	18	23	79	0.143	1.626	1.454	0.128	3.97	0.20	0.24	0.26
	3	161	176	15	28	36	92	0.146	1.226	1.200	0.244	4.11	0.15	0.20	0.19
High starch	6	164	192	28	32	40	96	0.100	1.747	1.218	0.118	4.45	0.16	0.23	0.26
	2	131	224	93		40	96	0.090	1.718	1.406	0.100	4.52	0.21	0.25	0.27
Carbohydrate-free	5‡	153	144	−9	2	3	64	0.121	0.977	1.317	0.115	3.88	0.10	0.32	0.30
	3	150	168	18		4	65	0.102	1.450	1.775	0.084	4.16	0.17	0.33	0.24

* Rats killed by stunning 24 hours after last treatment of CCl_4.

† Rats very deeply anesthetized. Marked intestinal hemorrhages present.

‡ Two other rats died within 12 hours after second treatment. No changes in the organs, except fatty liver, observed at necropsy.

difference in concentration of this vitamin in the liver and gut depended on the amount present in the latter organ. The marked difference in concentration of ascorbic acid in these two organs of rats fed the carbohydrate-free diet and subjected to carbon tetrachloride fumes indicate that the gut is the site of synthesis of vitamin C. The ascorbic acid is then absorbed and utilized by the liver.

SUMMARY

1. Rats are able to synthesize vitamin C irrespective of the composition of the diet. Adequate amounts of vitamin B factors are essential to ob-

[1] In a private communication, Doctor Higgins, Mayo Clinic, Rochester, Minn., has also noted hypertrophy of the adrenals under like conditions.

tain normal values for the concentration of vitamin C in the organs of the rat.

2. The utilization of ascorbic acid by the tissues of the rat is increased as the metabolic condition of the animal, under the influence of desiccated thyroid gland or α-dinitrophenol, is increased. The simultaneous administration of sodium fluoride and thyroid gland, at a level where either substance alone has no appreciable effect on the animal, decreases the vitamin C content of the organs. Rats at the point of collapse as the result of excessive thyroid feeding have a very low ascorbic acid content of the tissues.

3. The vitamin C content of the liver is decreased by the administration of cincophen or etherization of the animals.

4. The relative difference in concentration of ascorbic acid in the gut (small intestine) and liver of rats fed various diets indicates the important rôle played by the small intestine in yielding primary precursors for the synthesis of vitamin C. The adaptability of the gut to adjust itself to cope with experimental conditions, not normal to the ordinary life of the animal, as well as with variation in age is emphasized.

5. Rats with fatty livers and possible disarrangement of hepatic function, as the result of exposure to carbon tetrachloride vapor, still synthesize appreciable amounts of ascorbic acid.

REFERENCES

(1) Svirbely, J. L. Biochem. J. **27:** 960, 1933.
(2) Bessey, O. A. and C. G. King. J. Biol. Chem. **103:** 687, 1933.
(3) Harris, L. J. In J. M. Luck, Annual Review of Biochemistry, Stanford University Press, **4:** 350, 1935.
(4) Ray, S. N. Biochem. J. **28:** 996, 1934.
(5) Guha, B. C. and A. R. Ghosh. Nature **135:** 234, 1935.
(6) Euler, H. v., C. Gartz and M. Malmberg. Biochem. Ztschr. **282:** 399, 1935.
(7) Woodward, G. E. Biochem. J. **29:** 2405, 1935.
(8) Vars, H. M. and J. J. Pfiffner. Proc. Soc. Exper. Biol. Med. **31:** 839, 1934.
(9) Haworth, W. N. and E. L. Hirst. Chemistry and Industry **52:** 645, 1933.
(10) Bollman, J. L., F. C. Mann and M. H. Power. This Journal **111:** 483, 1935.
(11) Hopkins, F. G., B. R. Slater and G. A. Millikan. Biochem. J. **29:** 2803, 1935.
(12) Harris, L. J. and S. N. Ray. Biochem. J. **27:** 2006, 1933
(13) Zilva, S. S. Biochem. J. **29:** 2366, 1935.
(14) Svirbely, J. L. J. Biol. Chem. **111:** 147, 1935.
(15) Allers, W. D. Proc. Staff Meetings of Mayo Clinic **10:** 406, 1935.
(16) Phillips, P. H. and Chang Y. Chang. J. Biol. Chem. **105:** 405, 1934.
(17) Phillips, P. H., F. J. Stare and C. A. Elvehjem. J. Biol. Chem. **106:** 41, 1934.
(18) Phillips, P. H., H. E. English and E. B. Hart. This Journal **113:** 441, 1935.
(19) Bollman, J. L. and F. C. Mann. Ann. Int. Med. **9:** 617, 1935.

STUDIES ON THE VITAMIN A REQUIREMENTS OF THE RAT[1]

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SECTION I. During the course of experiments which were carried out on choledochocolonostomized adult rats maintained on the stock diet,[3] it was noted that the animals showed evidences of vitamin A deficiency, whereas the unoperated animals did not. This fact was established both on the basis of the vaginal smear picture and the depletion of the vitamin A stores in the liver, as determined by the antimony trichloride test at the time when the animals were sacrificed. At a time when the vaginal smear picture had been, for some time, that of a vitamin A deficient rat, administration of cod liver oil brought the picture back to that of a normal animal. The present experiments were carried out in order to throw further light on the cause of this phenomenon.

Adult rats which previously had been maintained on the stock ration were placed on a vitamin A deficient diet,[4] and the times required to deplete their vitamin A reserves, based on the vaginal smear pictures, were determined. In certain of these rats internal bile fistulas (1) were made, while the remainder were not subjected to operative procedure. Autopsies were performed on all of the animals, and only data obtained from those which showed a normal macroscopic picture were used. The bile fistula operations were likewise checked on autopsy.

The depletion times of this group of animals are given in table 1, series I. The reserves of vitamin A of the bile fistula group were depleted after a period varying from 43 to 63 days, while a period varying from 79 to 136 days was required to deplete the vitamin A reserves of the normal group. At the time of depletion each rat received 375 units of vitamin A

[1] We are indebted to E. R. Squibb and Sons for the fellowship which made this experimental work possible.

[2] E. R. Squibb and Sons Fellow, 1934–37.

[3] The stock diet consisted of ground wheat, 30 lbs.; fish meal, 5 lbs.; milk powder, 5 lbs.; alfalfa meal, 1 lb.; sodium chloride and calcium carbonate, 100 grams each. Lettuce was fed twice a week.

[4] The vitamin A deficient diet consisted of alcohol-extracted casein, 18 parts; starch, 68 parts; powdered yeast, 10 parts; Osborne and Mendel salt mixture, 4 parts. This was supplemented with 1 part of irradiated yeast or an adequate amount of viosterol as a vitamin D source. The latter substance was injected subcutaneously.

as cod liver oil by stomach tube. At the end of an 8 to 15 day period the bile fistula group again showed signs of depletion, while a period of 20 to 30 days was required to deplete the control group. Analogous results were obtained when the experiment was repeated a third time. The data indicate that vitamin A is much less effective, as judged by the depletion time,

TABLE 1

*Comparative vitamin A depletion times of bile fistula and normal rats**

RAT NUMBER		TIME NECESSARY TO DEPLETE INITIAL VITAMIN A RESERVES		TIME NECESSARY TO DEPLETE RESERVES AFTER FIRST VITAMIN A FEEDING		TIME NECESSARY TO DEPLETE RESERVES AFTER SECOND VITAMIN A FEEDING		TIME NECESSARY TO DEPLETE RESERVES AFTER THIRD VITAMIN A FEEDING		TIME NECESSARY TO DEPLETE RESERVES AFTER FOURTH VITAMIN A FEEDING	
Bile fistula	Normal	Bile fistula	Normal	Bile fistula	Normal	Bile fistula	Normal	Bile fistula	Normal	Bile fistula	Normal
Series I. Adult rats reared on the stock diet and then placed on a vitamin A free diet											
		days	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
1	5	45	103	15	20		24				
2	6	52	86	8	29		29				
3	7	43	79	10	30	11	23				
4	8	63	136		21		22				
	9		83		28		18				
	10		83								
Series II. Young growing animals reared on a vitamin A free diet											
11	16			13	19	13	20	15	25	14	23
12	17			22	28	15	19		27		24
13	18			14	29		25		24		
14	19			15	31		31		27		
15	20			14	31						
	21				26						
	22				31						
23	26			33†	62†	12	28	14	29		
24	27			40‡	61‡						
25				44‡							

* Unless otherwise stated, all animals received 375 units of vitamin A orally.

† Animal received 564 units of vitamin A orally.

‡ Animal received 620 units of vitamin A orally.

in the bile fistula than in the normal rat. From these data it is not possible to evaluate the factor of absorption.

A similar series of experiments was carried out on young growing rats. The animals were weaned when they were 28 days of age, and were then placed on a vitamin A free diet until their stores were depleted. Certain of the animals were given 375 units of vitamin A in the form of cod liver oil, by stomach tube; others received, respectively, 564 and 620 units.

The bile fistula operation was carried out 4 days later. The results are given in table 1, series II. Since vitamin A was administered before the operation, the factor of absorption was the same in both groups of rats. The vitamin A depletion times of the bile fistula animals were considerably less than those of the unoperated rats. This difference was shown on depleting both groups of animals, then administering cod liver oil, and again determining the times of depletion. No consistent results were obtained when the cod liver oil was injected subcutaneously or intraperitoneally.

A rapid loss in body weight followed shortly after the onset of the appearance of a straight cornified cell picture in the group of growing bile fistula rats. This could be checked and the weight curve could be made to rise again by the administration of vitamin A. Previous work had indicated that the administration of small doses of vitamin A to the depleted animals 1 or 2 days before the operation greatly prolonged the survival period and decreased the mortality rate.

The following experiments were carried out in order to evaluate the effect of operative procedure on the vitamin A content of the livers of rats. Three groups of animals were maintained on the stock diet, which was supplemented with 3500 units of vitamin A in the form of cod liver oil, over a 5 week period. The animals in the 3 groups were so distributed with respect to body weights that the average body weight (about 200 gms.) of the 3 groups was comparable. The first group was maintained as control. A laparotomy operation was performed on the members of the second group. Choledochocolonostomy operations were carried out on the members of the third group.

Five days after the operations all of the animals were sacrificed and the vitamin A content in the livers of the members in each group was determined. The average weight of the livers of 15 rats in the control group was 8.6 grams, and the average vitamin A content, in blue units, was 158. The average weight of the livers of 10 laparotomized rats was 7.6 grams, and the average vitamin A content was 149 blue units. Taking the average vitamin A content of the livers of the control group as unity, the average loss in vitamin A content of the livers of the laparotomized group was 6 per cent. The average weight of the livers of 14 choledochocolonostomized rats was 8.7 grams, and the average vitamin A content was 147 blue units. Using the previous method of calculation, the average vitamin A loss of this group was 7 per cent. The difference in the values for the average losses is less than the error in the method of estimating vitamin A. It is evident that the vitamin A content in the rat livers was not markedly influenced by the two operations.

The effect of icterus on the vitamin A requirements of the rat was determined. For this purpose, 20 young rats, which had been depleted of

their vitamin A reserves, were given 375 units of vitamin A in the form of cod liver oil, by stomach tube. Four days later the bile duct was ligated and sectioned at a point near the liver. The average time required for the animals to show evidence of vitamin A deficiency was 20 days. The average depletion time of 4 unoperated rats was 20.5 days. During this period marked liver damage had taken place as the result of the icterus, yet the vitamin A depletion times of these animals were not markedly reduced. It appears probable that the increased vitamin A requirements of the bile fistula rats are not due primarily to liver damage.

Fifteen members of this group of icteric animals were given each 375 units of vitamin A at the time when they showed evidence of vitamin A depletion. The vaginal smears of 2 members of this group returned to normal, one on the second day, and the other on the fifth day. The remainder showed a straight cornified cell picture for periods of 6 to 10 days following the administration of vitamin A. A third dose of 375 units of vitamin A was administered daily to each of the 7 animals which had not responded previously. One of the rats responded on the fourth day, while 4 others did not respond within a 10 day period. Two animals died on the fourth and fifth day, respectively, without the vaginal smear picture having returned to normal. Three of the rats which did not respond were then given 10,000 units of vitamin A orally in the form of halibut liver oil. The vaginal smear picture of 2 became normal within 3 days, while the third died after 4 days without the vaginal smear picture having become normal, although there were small amounts of vitamin A present in the liver. The depletion times of the 2 icteric rats which had received 10,000 units of vitamin A were 13 and 15 days, respectively. On administering 375 units of vitamin A to 6 vitamin A depleted, unoperated rats, the vaginal smear picture of all became normal within a period of 2 to 5 days.

These experiments indicate that, although vitamin A is absorbed from the intestinal tract of the jaundiced rat, the amount of a given dose absorbed is apparently markedly decreased over that in the control animal. It is therefore necessary, in order to restore the vaginal smear picture in the jaundiced rat to normal, to administer a considerably larger oral dose of vitamin A than to normal rats.

A possible explanation for the increased need of vitamin A by bile fistula rats may perhaps be due to excretion of the vitamin through the bile. However, experimental evidence did not support this view. Acidified, alkaline, and neutral samples of both rat and dog bile obtained from external bile fistula animals were extracted with petroleum ether.[5] The content of vitamin A, as determined by the antimony trichloride test, was

[5] Alcohol was used to break the emulsion.

either none or not more than a trace. No vitamin A was found in beef bile. The reliability of the procedure used to determine the presence of vitamin A in bile was established by the addition of vitamin A to beef bile and to a bile salt mixture. It was possible to recover about 70 per cent of the vitamin A which had been added.

Further evidence of the fact that bile contains little or no vitamin A was obtained from feeding experiments. Eight vitamin A depleted rats were given 2 cc. of ox bile orally daily for periods varying from 7 to 21 days. The vaginal smear pictures of none of these animals returned to normal. All of them showed a loss in body weight, and 7 of the 8 animals died during the course of the experiment. Daily doses of 5 cc. proved toxic. The vaginal smear pictures of 6 vitamin A deficient rats did not return to normal when they were fed a petroleum or ethyl ether extract[5] of 30 cc. of ox bile in olive oil daily for periods of 10 days to 5 weeks. All of the animals lost weight. Five of the animals died during the course of the experiment.

A group of rats with external bile fistulas, prepared according to Sawyer and Lepkovsky (2), were injected intravenously with *a*, 500 units of vitamin A in the form of an emulsion of a non-saponifiable fraction of halibut liver oil in a glucose-gelatin solution, or *b*, 500 units of beta carotene (2 mgm.) similarly emulsified. The bile was collected for a period of about 36 hours. Only traces of vitamin A were recovered, and none of the carotene. These findings check with previous reports that bile contains no appreciable quantities of vitamin A (3).

Discussion. The above experiments indicate that the choledochocolonostomized rat is depleted of its vitamin A stores considerably sooner under the conditions of the present experiments than the normal rat. This cannot be attributed to the effects of the operation itself, to liver damage, or to loss of vitamin A via the bile. Furthermore, it is not due entirely to decreased absorption of vitamin A from the intestinal tract, since the animals which had received vitamin A prior to the establishment of the bile fistulas were depleted of their vitamin A stores sooner than the unoperated animals. On the basis of the depletion times of the bile fistula rats as compared with the control animals, the amount of vitamin A which was absorbed was not less than 60 per cent of the doses which were administered.

The depletion times of icteric rats which had received vitamin A before the operation were not essentially different from those of normal rats despite the fact that in icteric rats there is extensive liver and other tissue damage. It was found necessary to administer considerably larger doses of vitamin A to vitamin A depleted icteric than to unoperated vitamin A depleted rats. It is not possible at this time to state whether the decreased net utilization of orally administered vitamin A by the icteric group is due

to a decrease in absorption or to a decreased utilization by the other damaged tissues of the body. The fact that the depletion times of the icteric rats which had received vitamin A before the operation were not markedly altered as contrasted with the normal group indicates that the decreased depletion time, when vitamin A was administered after the operation, was probably due to decreased absorption.

The data do not indicate in a positive way the reasons for the more rapid depletion of vitamin A in the bile fistula rats.

Bile fistula rats do not become anemic. The spleens are very often small. Some of the rats, especially the males, when maintained on a high sugar diet, often bleed spontaneously from the intestinal tract. Some of the animals exhibit decreased coagulability of the blood. This condition usually does not develop until several weeks after the bile fistula operation. Bile feeding tends to correct the condition. In some cases, although not invariably so, ulcers are present in the intestinal tracts. The site is not related to the site of the operation. Some of the rats show edema in the tissues of the neck, especially in the lymph nodes. The lungs are often a bright red color. Hawkins and Whipple (4) have reported somewhat similar findings in bile fistula dogs.

SECTION II. *Some aspects of the vitamin A requirements of the rat.* The experiments which are described below were carried out in order to determine what effects, if any, certain factors, such as age, growth, ovariectomy and thyroidectomy, have on the vitamin A requirements of the rat.

Young female rats, which had been weaned at 28 days of age, were reared on a vitamin A free diet until, on the basis of their vaginal smears, their vitamin A stores were depleted. Each animal was then given 375 units of vitamin A, in the form of cod liver oil, by stomach tube. The operations were performed 4 days later. Insofar as possible, the rats were divided so as to distribute litter mates between control and experimental groups. For purposes of serving as controls in the successive experiments, since it was impossible to carry out all of the experiments simultaneously, certain of the animals which had served as controls in one experiment, after depletion of their stores, were given vitamin A and were used as controls in the next experiment. All of the animals had the same nutritional history and were approximately of the same age. All of the data which were obtained in each of the experimental series have, for statistical purposes, been treated as a whole and conclusions have been drawn therefrom.

The data for the various groups of normal control animals are given in table 2. It is evident that the time required to deplete the vitamin A stores of the rat, when the given dose of vitamin A was administered, is, within the limits of the ages and body weights of the animals studied, independent of these factors. These data check with the data which were

obtained in section I of this paper. The average age of the normal adult rats reported in the first section of this paper was about 10 months, and

TABLE 2

Relation of body weights and ages of avitaminosis A rats to the depletion time following administration of 375 units of vitamin A

STATISTICAL CONSTANTS (CALCULATED)	NUMBER OF ANIMALS	AGE	BODY WEIGHT AT BEGINNING OF EXPERIMENT	WEIGHT GAIN*	MAXIMUM WEIGHT GAIN	DEPLETION TIME	TIME TO ATTAIN MAXIMUM WEIGHT	DAILY WEIGHT GAIN*	DAILY WEIGHT GAIN TO MAXIMUM WEIGHT	WEIGHT GAIN PER 100 VITAMIN UNITS*	WEIGHT GAIN PER 100 VITAMIN UNITS TO MAXIMUM WEIGHT
				Series I							
		weeks	*grams*	*grams*	*grams*	*days*	*days*	*grams*	*grams*	*grams*	*grams*
Average values	43	10	117.3	36.6	41.4	24.1	29.6	1.51	1.45	9.8	11.1
σ				10.85	13.03	2.07	4.89	0.41	0.34	3.0	3.1
P. E.†				7.32	8.79	1.39	3.30	0.28	0.23	2.0	2.1
P. E. of mean				1.13	1.36	0.22	0.51	0.04	0.05	0.31	0.32
				Series II							
Average values	24	15	154	20.4	25.5	24.7	29.7	0.83	0.86	6.3	6.8
σ				7.81	8.21	4.52	5.73	0.37	0.35	1.9	2.3
P. E.				5.27	5.53	3.04	3.86	0.25	0.24	1.3	1.5
P. E. of mean				1.09	1.15	0.63	0.81	0.05	0.05	0.27	0.32
				Series III							
Average values	8	20	171	10.6	12.4	24.8	25.1	0.45	0.49	2.9	3.5
σ				1.99	3.04	3.86	6.09	0.13	0.10	0.5	0.8
P. E.				1.34	2.05	2.60	4.11	0.09	0.07	0.3	0.6
P. E. of mean				0.51	0.78	0.98	1.55	0.03	0.03	0.13	0.21
				Series IV							
Average values	6	25	181	6.6	7.4	23.2	23.0	0.35	0.43	2.0	2.0
σ				2.84	2.72	3.97	3.52	0.21	0.21	0.7	0.7
P. E.				1.91	1.84	2.68	2.37	0.14	0.14	0.5	0.5
P. E. of mean				0.86	0.82	1.19	1.06	0.06	0.06	0.22	0.22
				Series V and VI							
Average values	3	30–39	183	10.0	11.0	23.6	25.3	0.42	0.42	2.5	2.8

* These values cover the period from the time of vitamin A administration to the time of vitamin A depletion based on the vaginal smear picture.

† P. E. = probable error.

the average body weight was 230 grams. The average time required to deplete the vitamin A reserves, when a dose of 375 units was administered,

was 23.5 days, with a standard deviation of 2.74, a probable error of 1.85, and the probable error of the mean of 0.62 day.

The procedure employed to study the relation of the ovary to the vitamin A requirements of the rat was essentially the same as was used in the other experiments which are described in this paper. Vitamin A was administered 4 days prior to the operation. The time required to deplete the vitamin A stores of the rats subsequent to bilateral ovariectomy or ovary transplantation in ovariectomized rats[6] was determined. The restoration of the sexual cycle was used as evidence of successful ovarian transplantation. A group of unoperated rats and a group of rats on which a laparotomy was performed were used as experimental controls. The data are given in table 3, series I. The data obtained in series II of this table were obtained by depleting the vitamin A reserves of these animals, then administering 375 units of vitamin A, and again determining the depletion time. The calculations are given as per cent of the values which were obtained on the normal group of animals.

The data indicate that, within the limits of error in the methods employed, the ovary is without influence on the vitamin A requirements of the rat. It is noteworthy that the weight increment, per 100 vitamin A units, in both of the experimental series, is greater in the ovariectomized group than in the other groups of rats in the first experimental series. This indicates that the added weight of the animals did not increase their vitamin A requirements. On the other hand, the effect of ovarian transplantation is to decrease somewhat the weight increment per 100 vitamin A units without, however, materially affecting the total vitamin A requirements of the rats. This effect can be attributed to the operative procedure since the same effect is shown by series I of the laparotomized group. This conclusion is substantiated by the fact that normal values were obtained in the second series in both the laparotomized and in the ovary transplant group.

The uteri of the unoperated and ovarian transplant groups which had been maintained on low levels of vitamin A were markedly hypertrophied, while the uteri of the ovariectomized rats which had been maintained on a similar diet were atrophied. This indicates that the hypertrophy of this organ is dependent, in vitamin A deficiency, upon the ovary.

For purposes of studying the influence of thyroid activity and of dinitrophenol on the vitamin A requirements of the rat, young vitamin A depleted rats of 80 to 120 grams body weight were given 375 units of vitamin A in the form of cod liver oil by stomach tube. The animals were divided into groups which have been designated in table 3. The thyroid

[6] The ovaries were transplanted subcutaneously or next to the thyroid gland. The vaginal smear picture was not influenced by the technique used in taking the smears.

TABLE 3

*Summary of experimental data relating to various groups of rats**

ANIMAL GROUP	EXPERIMENTAL SERIES NUMBER	NUMBER OF ANIMALS IN GROUP	AVERAGE INITIAL BODY WEIGHT	AVERAGE AGE AT BEGINNING OF EXPERIMENT	AVERAGE WEIGHT GAIN†	AVERAGE WEIGHT GAIN TO MAXIMUM WEIGHT	AVERAGE DEPLETION TIME†	AVERAGE TIME TO ATTAIN MAXIMUM WEIGHT	AVERAGE DAILY WEIGHT GAIN†	AVERAGE DAILY WEIGHT GAIN TO MAXIMUM WEIGHT	AVERAGE WEIGHT GAIN PER 100 VITAMIN UNITS†	AVERAGE WEIGHT GAIN PER 100 VITAMIN UNITS TO TIME OF MAXIMUM WEIGHT
			grams	*weeks*	*(per cent of normal)*††	*(per cent of normal)*	*(per cent of normal)*	*(per cent of normal)*	*(per cent of normal)*	*(per cent of normal)*	*(per cent of normal)*	*(per cent of normal)*
Normal	1	53	117.3	10	100, ±3, ±3	100, ±3, ±3	100, ±1, ±1	100, ±2, ±2	100, ±3, ±3	100, ±3, ±3	100, ±3, ±3	100, ±3, ±3
Normal	2	34	154.0	15	100, ±5, ±5	100, ±4, ±4	100, ±3, ±3	100, ±3, ±3	100, ±6, ±6	100, ±6, ±6	100, ±5, ±5	100, ±4, ±4
Laparotomy	1	8	104.5	10	82, ±5, ±4	88, ±4, ±3	99, ±2, ±2	100, ±2, ±2	83, ±7, ±6	86, ±4, ±3	81, ±5, ±4	89, ±3, ±3
Laparotomy	2	6	137.6	15	109, ±10, ±10	101, ±5, ±5	97, ±2, ±2	94, ±2, ±2	112, ±7, ±8	108, ±7, ±7	94, ±7, ±7	102, ±5, ±5
Ovariectomy	1	33	113.5	10	135, ±3, ±4	137, ±3, ±4	108, ±3, ±3	108, ±2, ±2	125, ±3, ±4	122, ±3, ±4	140, ±3, ±5	136, ±3, ±4
Ovariectomy	2	18	164.3	16	135, ±5, ±6	121, ±4, ±5	103, ±2, ±2	100, ±3, ±3	131, ±6, ±8	121, ±5, ±6	119, ±7, ±8	121, ±5, ±6
Ovary transplant	1	9	112.9	10	73, ±4, ±3	83, ±6, ±5	101, ±5, ±5	95, ±3, ±3	72, ±7, ±5	85, ±5, ±4	72, ±4, ±3	83, ±6, ±5
Ovary transplant	2	7	143.7	15	96, ±9, ±9	91, ±7, ±6	96, ±4, ±4	92, ±4, ±4	100, ±11, ±11	99, ±7, ±7	83, ±9, ±8	91, ±7, ±6
Thyroidectomy	1	10	114.1	10	54, ±10, ±5	52, ±12, ±5	156, ±3, ±5	125, ±6, ±7	34, ±7, ±2	39, ±11, ±3	53, ±10, ±4	52, ±11, ±5
Thyroidectomy	2	5	132.4	17	91, ±7, ±6	74, ±10, ±7	141, ±4, ±6	105, ±6, ±6	52, ±6, ±3	69, ±5, ±4	71, ±8, ±6	70, ±10, ±7
Thyroidectomy plus desiccated thyroid tissue‡	1	3	119.1	10	11	24	114	103	18	23	11	15
Thyroidectomy plus desiccated thyroid tissue§	2	4	128.2	15	49	57	98	94	59	65	52	63
Normal plus thyroxine¶	1	6	118.3	10	67, ±9, ±6	64, ±8, ±5	102, ±4, ±4	91, ±5, ±5	65, ±5, ±3	69, ±6, ±4	66, ±8, ±5	63, ±9, ±6
Normal plus thyroxine‖	2	6	140.1	15	30, ±10, ±4	34, ±14, ±5	68, ±4, ±3	69, ±6, ±4	44, ±6, ±3	50, ±7, ±4	25, ±10, ±3	34, ±11, ±3
Normal plus dinitrophenol**	1	12	124.0	10	54, ±10, ±5	58, ±14, ±9	83, ±5, ±4	77, ±8, ±6	66, ±9, ±6	71, ±8, ±6	54, ±9, ±5	54, ±10, ±5
Normal plus dinitrophenol**	2	11	148.0	15	65, ±9, ±6	59, ±12, ±7	97, ±3, ±3	70, ±6, ±4	72, ±8, ±6	82, ±9, ±7	56, ±8, ±5	58, ±9, ±6
Normal plus dinitrophenol‡‡	1	6	126.0	10	−56, ±9, ±5		81, ±4, ±3		−71, ±7, ±5			

* All of the animals received 375 units of vitamin A as cod liver oil by stomach tube at the beginning of the experiment.

† These values cover the period from the time of vitamin A administration to the time of vitamin A depletion, based on the vaginal smear picture.

‡ Five-tenths gram of desiccated thyroid tissue was administered orally on the 5th, 13th, and 21st day of the experiment.

§ Five-tenths gram of desiccated thyroid tissue was administered orally on the 3rd, 14th, 18th, and 24th day of the experiment.

¶ Two-tenths milligram thyroxine was administered intraperitoneally on the 3rd, 5th, 11th, 16th, and 21st day of the experiment.

‖ Two-tenths milligram thyroxine was administered on the 4th and 6th and 0.5 mgm. on the 11th, 15th, 17th, and 20th day of the experiment.

** Twenty milligrams of dinitrophenol per kilo of body weight were given subcutaneously twice daily beginning on the 4th day of the experiment.

†† The first ± figure in each of the columns is the probable error in terms of the value for the experimental group. The second ± figure is the probable error in terms of the value for the corresponding normal group of the respective series.

‡‡ Twenty-three and eight-tenths milligrams (average) of dinitrophenol per day were ingested with the diet.

operation was carried out 4 days after administering vitamin A. Part of this group of animals received desiccated thyroid tissue orally, while the rest did not. The thyroid tissue was found to be free from vitamin A. The data given in series II of the thyroid experiments were obtained by depleting the rats of the first series of their vitamin A stores and subsequently administering 375 units of vitamin A orally. Administration of desiccated thyroid tissue was discontinued for several days before the second dose of vitamin A was given.

The data given in table 3 indicate that the removal of the thyroid gland markedly prolonged the vitamin A depletion time. The body-weight gain was markedly reduced both in absolute amount and when calculated per 100 vitamin A units. The basal metabolic rate of the thyroidectomized rats was 30 to 40 per cent less than that of the normal animals. The peak of the weight curve was reached at approximately the same time as in the case of the normal group. Feeding of desiccated thyroid tissue in the stated doses to thyroidectomized rats led to a reduction of weight gain as compared with the control groups. This is due to the acceleration of the metabolic rate. The vitamin A depletion time was practically that of normal rats. The effect on the weight gain of the unoperated group of rats which were given thyroxine varied somewhat depending on the length of time during which thyroxine accelerated the metabolic rate. In the first series the depletion time was normal due to the fact that there is a considerable latent period following thyroid administration. The dosage was increased in the second series. The thyroid effects were cumulative. The vitamin A depletion time was markedly reduced.

Under the conditions of the experiment, dinitrophenol did not markedly influence the vitamin A depletion times despite its well known effect in raising the basal metabolic rate. This is especially shown in the case of those animals which received dinitrophenol orally and in which the weight loss was considerable.

Discussion. The data indicate that, within the limits of age and body weights of the rats used, the vitamin A requirements are independent of these factors. The meaning of this is not entirely clear. On the basis of these data, the vitamin A requirements cannot be strictly proportional to the amount of tissue of the animal. This, of course, would be anticipated, since fluids, the skeleton, adipose tissue, and glycogen stores, which contribute to the body weight, probably do not require vitamin A. On the other hand, the vitamin A requirements cannot be assumed to be strictly proportional to the amount of such a tissue as muscular tissue, since the amount of the latter must vary greatly with the weight and age of the animal. Possibly, although this has not been proved, the vitamin A requirements may depend on the amount of nervous tissue. Although this

may vary somewhat with the age and weight of the animal, the variation is probably less than that of the amount of the other tissues.

The findings that the vitamin A requirements are independent of ovarian function are in agreement with the results of Mason and Wolfe (5), who report that complete castration led to no significant difference in the time of appearance of continuous cornification or of xerophthalmia in female vitamin A deficient rats. The present experiments are an extension of this work. In connection with the present data, the results of Kudrjashov (6) are of interest. His data indicate that the absence of vitamin A has no hindering influence on the synthesis of follicular hormone.

The present data point to an interrelationship between the thyroid hormone and vitamin A. This is in harmony with the previous reports of von Euler and Klussmann (7), Fasold and Peters (8), Eufinger and Gottlieb (9), Abelin (10), Mitzkewitch (11), Coplan (12), and Parhon and Werner (13). The nature of this interrelationship is not clear. On the basis of the dinitrophenol experiments, it seemingly is not a question of metabolic rate. However, it must be borne in mind that the action of dinitrophenol and that of thyroid hormone are, in many respects, not comparable. A possible explanation is that the antagonism between the two substances is confined to the nervous system. This has not been proved. Possible support for this hypothesis may perhaps be found in the results of Mellanby (14) and others (15), which show that avitaminosis A is followed by characteristic changes in the nervous system. The thyroid hormone likewise appears in part to act through the nervous system (16). However, until further data are obtained on these subjects, any hypothesis must be regarded as wholly speculative.

SUMMARY

1. Experiments which are based on the depletion times of avitaminosis A rats after administration of given doses of vitamin A are described.

2. The data indicate: *a*. Laparotomy is without appreciable effect on the vitamin A requirements of the rat. *b*. The vitamin A requirements of choledochocolonostomized rats are greater than those of normal rats. *c*. The bile is not a channel for the excretion of vitamin A. *d*. The livers of choledochocolonostomized rats do not contain less vitamin A than unoperated rats maintained under the same conditions. *e*. The vitamin A requirements of icteric rats do not differ materially from those of normal rats. *f*. Although vitamin A, when administered by stomach tube to the icteric rat, is absorbed, the amount is less than in the case of the normal rat. *g*. The vitamin A requirements of the rat are, within the limits studied, independent of the age and the body weight. *h*. No relationship exists between the vitamin A requirements of the rat and the ovary. *i*.

The vitamin A requirements of the rat may be increased by administration of thyroxine or desiccated thyroid tissue. In thyroidectomized rats, the need for vitamin A is decreased.

REFERENCES

(1) Greaves, J. D. and C. L. A. Schmidt. J. Biol. Chem. **102:** 101, 1933. This Journal **111:** 492, 1935.
(2) Sawyer, L. and S. Lepkovsky. J. Lab. and Clin. Med. **20:** 958, 1935.
(3) von Drigalski, W. Ztschr. Vitaminforsch. **3:** 37, 1934.
Desanctis, M. Ann. d'igiene **33:** 771, 1923.
(4) Hawkins, W. B. and G. H. Whipple. J. Exper. Med. **62:** 599, 1935.
(5) Mason, K. E. and J. M. Wolfe. J. Nutrition **9:** 725, 1935.
(6) Kudrjashov, B. A. Arch. exper. Path. u. Pharmakol. **178:** 295, 1935.
(7) von Euler, H. and E. Klussmann. Ztschr. physiol. Chem. **213:** 21, 1932.
(8) Fasold, H. and H. Peters. Ztschr. ges. exper. Med. **92:** 57, 1933.
(9) Eufinger, H. and J. Gottlieb. Klin. Wchnschr. **12:** 1397, 1933.
(10) Abelin, I. Ztschr. physiol. Chem. **217:** 109, 1933; Ztschr. Vitaminforsch. **4:** 120, 1935.
(11) Mitzkewitch, M. S. Arch. exper. Path. u. Pharmakol. **174:** 339, 1934.
(12) Coplan, H. M. J. Nutrition **9:** 469, 1935.
(13) Parhon, C. and G. Werner. Compt. Rend. Soc. Biol. **118:** 1659, 1935.
(14) Mellanby, E. Nutrition and disease. Edinburgh, 1934. J. Path. **38:** 391, 1934; Brain **58:** 141, 1935; Edinburgh Med. J. **40:** 197, 1933.
(15) Sutton, T. S., H. E. Setterfield and W. E. Krauss. Ohio Agric. Exper. Sta. Bullet. 545, December, 1934.
Setterfield, H. E. and T. S. Sutton. J. Nutrition **9:** 645, 1935.
Aberle, S. B. D. J. Nutrition **7:** 445, 1934.
Zimmermann, H. N. J. Exper. Med. **57:** 215, 1933.
See also S. B. Wolbach and P. R. Howe. J. Exper. Med. **42:** 753, 1925.
(16) Mansfeld, G. and G. Horváth. Pflüger's Arch. **235:** 520, 1935.

STUDIES OF THE ENERGY OF METABOLISM OF NORMAL INDIVIDUALS: A STANDARD FOR BASAL METABOLISM, WITH A NOMOGRAM FOR CLINICAL APPLICATION

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This study is based on the determination of the energy of metabolism, under standard conditions, of 639 male and 828 female human subjects selected as normal individuals from a series of determinations made on more than 80,000 individuals, which is now in the course of statistical analysis. Preliminary reports have been previously published, but since these were issued, supplementary observations have been added to the normal series, and the whole data have been rechecked and reanalyzed. The results presented here constitute the definitive findings for this group of data.

The phrase "basal metabolism" as well as the German term "Grundumsatz" was of advantage in the early days of the investigation of the energy of metabolism in that it emphasized the necessity of carefully excluding all determinations not carried out under the strictly standard conditions which are intended to exclude the effects of the influence of food, previous exercise, muscular movement, mental and physical irritation, fear, restlessness, and the elevation of body temperature as well as extremes of environmental temperature. Unfortunately, the use of the term has led in recent years to the almost subconscious acceptance of the erroneous idea that, of any two or more determinations carried out on the same individual, the lowest or the lower group of such a series were the correct ones, and that the higher ones were vitiated by the presence of some unknown disturbing factor. Variability is a universal biologic phenomenon; it must be taken account of by proper statistical methods and not excluded by a predetermined selection of results. On account of the well established use of the terms we do not consider it advisable to change them, although we agree with Krogh that the term "standard metabolism," if it had been used originally, would have been preferable to "basal metabolism." We hope that our treatment of the subject in this series of papers will help to correct the erroneous notion that there is a single lowest "basal" me-

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tabolism for each individual, and to emphasize the fact that metabolism is a variable quantity that can be standardized, but not absolutely fixed.

The individuals included in the group were selected from the large clientele that were examined in the basal metabolism laboratory of The Mayo Clinic since 1917. They comprise two main groups: 1, the individuals up to about 17 years of age, who were mostly Rochester school children and were considered normal by the school physician, and 2, the adults, who were either Mayo Clinic employees, professional and non-professional, or patients at the clinic. For the selection of the patients in the adult group, the medical histories were examined carefully, and from them were chosen individuals whose symptoms and physical findings gave every reason to assume that they had no disturbance of metabolism. It should be explained that a great many individuals receive routine examinations in this institution, who have no particular complaint, but who have asked for a "checkup" examination. Others have only trivial symptoms, and others still, although subject to some organic lesions (for example, hernia, ocular defects, minor injuries) cannot be considered reasonably to have any disturbance of metabolism. The adult group consists of persons from all parts of the country and from the various social strata of their respective populations, and in that sense this group is fairly representative of the country as a whole. Unlike the adult group, the children were, in the main, born and brought up in Rochester, and therefore open to the criticism of being representative of only a particular locality. To offset this, in part, it should be pointed out that this group includes children from all the classes of the Rochester population, inasmuch as there are no private schools in this city. Also there is no endemic goiter at the present time in Rochester, so that this factor of influence on the level of metabolism does not affect our results. So far as doubting the "normality" of this series is concerned, other considerations, which it will be well to mention here, diminish the importance of whether particular individuals should have been allowed in this "normal" series. Our conclusions, as will appear later, rest on a systematic analysis of the series, in yearly age groups, and the homogeneity that appears in the data when studied in relation to age gives conviction to the results as a whole. Furthermore, the individuals on whom they are based had been examined thoroughly by a group of competent physicians who had not considered any one of them in any sense as presenting conditions that require attention for a disturbance of metabolism. The clinical application of the estimation of basal metabolism is to determine whether there is any evidence in this determination which would cause the physician to consider that he is dealing with a problem of altered metabolism. Our series, therefore, is appropriate for the standardization of metabolism for clinical purposes, and because of the good cross-section of the country population that it

affords, it also is appropriate for use in studying the broader problem of the effect of such factors as race, climate or geographical influence on the energy of metabolism.

The calculations used for the erection of our standards have been made from the first determination made for the individual, unless at the time of the test and before its calculation, it was noted as "unsatisfactory" for reasons of restlessness, observable nervous tension, or an elevated temperature. Even in cases in which several observations were made either on the same or different days, the first only was used for this study. In consequence, the element of training is excluded from our normal standards, even as it is in clinical application or as it would of necessity be in a comparative study of any large group of individuals. It would be beside the point to define a physiologic normal which was set up under conditions which might appear theoretically ideal, and then apply that standard to the different conditions which must obtain in nearly any type of investigation which utilizes a large number of individuals. If many determinations are made on an individual and, of these, either the mean or lowest is used, the results will depend on the number of determinations which have been made for each case. Where a mean of several determinations is used, the value is slightly lower than the mean of first determination for many similar individuals, and of course this is true for the lowest determination. Therefore, if one departs from the practice of using a single determination made under standard conditions, the number of determinations should strictly be identical for each individual. That this condition would limit most investigations to few individuals is obvious, and therefore we consider that a standard should be built strictly on the single determination.

The method of determining the metabolism was the gasometer or open circuit method with duplicate analysis of the expired air, as described in detail by Boothby and Sandiford (2). Each subject came to the laboratory in the morning, without breakfast, from his residence which was never more than a mile and a half from the clinic, and before the test, rested for half an hour, horizontally on a cot. The use of the gasometer method precludes any exercise of arbitrary judgment or prejudice, such as frequently enters into the drawing of the line from which the metabolism is calculated, when a closed circuit type of apparatus is used. The gasometer method also greatly decreases the magnitude of the fallacious result obtained if, during the experimental period, the subject allows the average level of his "reserve" or "supplementary" respiratory volume to change. The gas analyses were executed in duplicate by trained technicians under carefully supervised conditions, in a laboratory and with a staff organized to do more than fifty metabolic determinations daily.

In this paper the results will be expressed in terms of calories per hour

TABLE 1

Basal metabolism, calories per square meter (DuBois) per hour, means, and standard deviations, observed, for age in years to nearest birthday

AGE NEAREST BIRTHDAY	CALORIES PER SQUARE METER PER HOUR					
	Males			Females		
	Number	Mean	Standard deviation	Number	Mean	Standard deviation
years						
6	23	53.26	3.55	21	50.69	3.35
7	22	52.48	2.74	24	50.09	2.50
8	27	51.86	2.64	25	45.92	2.33
9	24	50.98	3.18	20	46.37	2.77
10	23	48.34	3.01	20	45.92	3.08
11	22	47.70	3.03	26	46.07	3.16
12	33	46.94	3.12	27	43.98	2.92
13	21	45.47	4.22	17	43.55	2.96
14	24	46.55	3.42	16	42.04	3.38
15	29	46.21	3.29	21	39.99	2.94
16	26	46.08	2.40	21	39.60	3.15
17	18	44.78	3.35	4	37.35	1.58
18	4	44.95	1.28	9	37.33	2.73
19	11	41.79	1.81	30	35.70	2.68
20	4	42.52	1.60	18	36.73	2.65
21	10	40.96	2.51	23	36.27	3.10
22	3	42.27	1.23	29	35.72	3.25
23	5	39.46	1.44	23	36.23	3.16
24	8	41.27	2.98	19	35.78	2.20
25	11	40.84	1.88	15	36.08	1.61
26	10	39.15	1.82	15	36.63	2.50
27	6	41.42	.94	13	36.83	1.92
28	5	40.98	2.55	15	36.41	2.59
29	8	41.19	2.61	12	35.29	1.47
30	9	40.00	3.79	15	35.33	2.06
31	5	39.72	3.39	9	35.63	2.94
32	12	40.31	2.06	12	35.35	2.16
33	7	38.51	2.14	15	35.93	2.13
34	5	38.00	1.40	15	35.30	2.33
35	15	39.19	2.09	7	35.78	1.08
36	12	37.66	2.08	10	35.92	1.88
37	2	38.75	.05	8	34.84	2.26
38	7	39.46	1.77	11	35.60	2.48
39	5	39.94	2.29	12	35.69	2.34
40	11	37.95	2.88	13	35.38	1.96
41	14	37.89	2.26	13	35.36	1.62
42	7	37.71	2.83	12	36.06	2.70
43	14	37.15	2.94	15	35.23	2.11
44	15	39.02	1.85	11	36.80	2.63
45	9	37.74	1.58	12	35.02	1.36
46	11	36.48	2.29	12	35.66	2.73

TABLE 1—*Concluded*

AGE NEAREST BIRTHDAY	CALORIES PER SQUARE METER PER HOUR					
	Males			Females		
	Number	Mean	Standard deviation	Number	Mean	Standard deviation
years						
47	8	38.50	2.56	6	34.87	2.04
48	5	35.00	2.17	11	33.80	2.46
49	9	37.42	4.02	8	35.11	2.19
50	9	36.89	2.41	16	33.91	1.78
51	8	37.29	1.40	9	32.58	1.14
52	15	36.29	2.94	13	34.50	1.96
53	3	35.70	3.06	14	34.44	2.33
54	6	35.43	2.51	12	34.13	2.24
55	5	35.52	1.92	12	33.24	1.74
56	6	35.95	3.27	6	32.87	2.28
57	6	34.67	2.44	10	32.76	2.01
58	3	37.07	2.23	7	33.98	1.41
59	6	38.03	1.21	6	34.32	1.79
60	5	35.22	1.71	6	34.68	2.46
61	3	36.93	1.83	2	32.35	1.15
62				7	34.07	1.89
63	2	37.30	2.91	3	30.73	2.21
64	3	37.77	2.24			
65				4	35.10	2.87
68				11	33.28	2.72

related to "surface area." Here surface area is estimated from stature and body weight by the formula of DuBois and DuBois

$$S.A. = 0.007184 \times W^{0.425} \times S^{0.725}$$

in which $S.A.$ is the surface area in square meters, W is the body weight in kilograms, and S is the stature in centimeters. In another paper we shall examine critically the standardization of basal metabolism on a linear formulary basis. Suffice it here to say that our conclusion is that no better method than the one of "surface area" is at hand and that this method has certain distinct advantages over the linear method. To avoid repetition hereafter, when we shall use in this text the phrase "surface area," it is to be understood that the surface area has been estimated by this formula.

The male and female series were analyzed separately. For each yearly age group as of nearest birthday[2] the mean and standard deviation of the

[2] Our data were assembled and analyzed in yearly groups on the basis of age at nearest birthday. However, since it is customary to express age as at last birthday, our final standards are so expressed.

metabolism, expressed as calories per square meter per hour were calculated. The results are given in table 1. In figure 1 the means and standard deviations are plotted with smooth curves drawn to the observed points. The smoothing of the curve of means was executed methodically and with care. With so complex a curve, it was impracticable to attempt to fit an algebraic function for the entire range of age by a method such as least squares. Instead, a method similar to that used for fitting

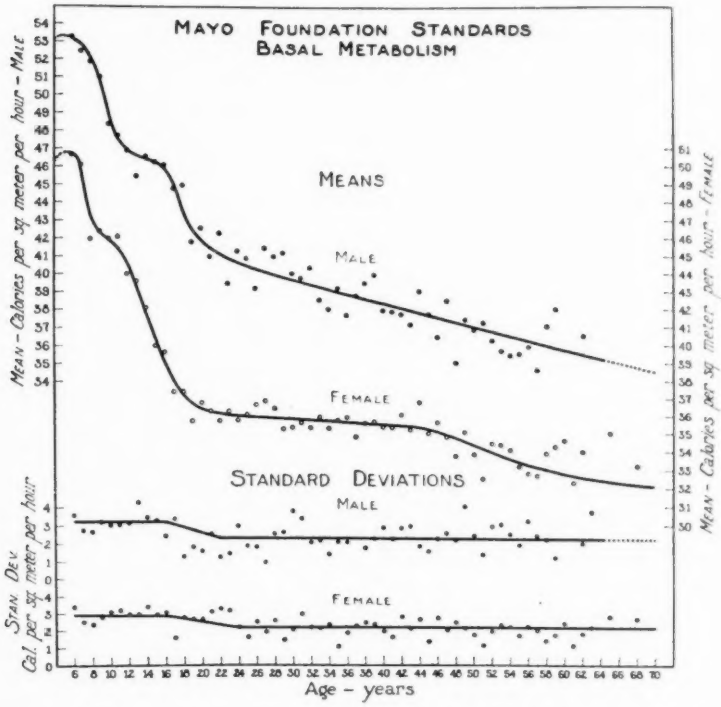

Fig. 1. Smooth curves drawn to observed means and standard deviations, calories per square meter (DuBois) per hour.

actuarial curves was utilized. Limited portions of the data were tentatively fitted with simple parabolic curves by least squares, and the entire series then fused graphically. The curve fitted to the standard deviations was obtained similarly, but since the variation of these was not large, as simple a curve as possible was drawn. The numerical values corresponding to the standard curve of means is given in table 2, as of age in whole years, last birthday. These values were obtained by reading from a

TABLE 2

Basal metabolism, mean calories per square meter (DuBois) per hour; smoothed values for age in whole years as of last birthday

AGE LAST BIRTHDAY	CALORIES PER SQUARE METER PER HOUR				AGE LAST BIRTHDAY	CALORIES PER SQUARE METER PER HOUR			
	Males		Females			Males		Females	
	Mean	Standard deviation	Mean	Standard deviation		Mean	Standard deviation	Mean	Standard deviation
years					*years*				
6	53.0	3.2	50.5	2.9	41	38.2	2.3	35.5	2.2
7	52.4	3.2	48.5	2.9	42	38.0	2.3	35.5	2.2
8	51.5	3.2	46.7	2.9	43	37.9	2.3	35.4	2.2
9	49.9	3.2	46.1	2.9	44	37.7	2.3	35.4	2.2
10	48.0	3.2	45.7	2.9	45	37.6	2.3	35.3	2.2
11	47.2	3.2	45.1	2.9	46	37.5	2.3	35.1	2.2
12	46.8	3.2	43.9	2.9	47	37.4	2.3	34.9	2.2
13	46.5	3.2	42.5	2.9	48	37.2	2.3	34.7	2.2
14	46.4	3.2	41.1	2.9	49	37.1	2.3	34.6	2.2
15	46.1	3.2	39.7	2.9	50	37.0	2.3	34.4	2.2
16	45.5	3.1	38.6	2.8	51	36.8	2.3	34.2	2.2
17	44.4	3.0	37.6	2.7	52	36.7	2.3	34.0	2.2
18	42.9	2.8	37.0	2.6	53	36.6	2.3	33.8	2.2
19	42.2	2.7	36.6	2.6	54	36.5	2.3	33.6	2.2
20	41.6	2.5	36.3	2.5	55	36.3	2.3	33.4	2.2
21	41.2	2.4	36.2	2.4	56	36.2	2.3	33.3	2.2
22	40.9	2.3	36.1	2.3	57	36.1	2.3	33.2	2.2
23	40.7	2.3	36.1	2.2	58	36.0	2.3	33.1	2.2
24	40.5	2.3	36.0	2.2	59	35.8	2.3	32.9	2.2
25	40.3	2.3	36.0	2.2	60	35.7	2.3	32.8	2.2
26	40.1	2.3	35.9	2.2	61	35.6	2.3	32.8	2.2
27	40.0	2.3	35.9	2.2	62	35.5	2.3	32.7	2.2
28	39.9	2.3	35.9	2.2	63	35.4	2.3	32.6	2.2
29	39.7	2.3	35.9	2.2	64	35.3	2.3	32.5	2.2
30	39.6	2.3	35.8	2.2	65	35.1*	2.3*	32.4	2.2
31	39.5	2.3	35.8	2.2	66	35.0*	2.3*	32.3	2.2
32	39.3	2.3	35.8	2.2	67	34.9*	2.3*	32.3	2.2
33	39.2	2.3	35.7	2.2	68	34.8*	2.3*	32.3	2.2
34	39.1	2.3	35.7	2.2	69	34.7*	2.3*	32.2	2.2
35	38.9	2.3	35.7	2.2	70	34.5*	2.3*	32.2	2.2
36	38.8	2.3	35.6	2.2	71	34.4*	2.3*	32.1*	2.2*
37	38.7	2.3	35.6	2.2	72	34.1*	2.3*	32.1*	2.2*
38	38.5	2.3	35.6	2.2	73	33.9*	2.3*	32.1*	2.2*
39	38.4	2.3	35.6	2.2	74	33.7*	2.3*	32.0*	2.2*
40	38.3	2.3	35.5	2.2	75	33.4*	2.3*	32.0*	2.2*

* Extrapolated.

graph of the curve drawn on finely divided paper. Since the values are to be applied to the age as of last birthday, they were read half way between the age indicated, and the next succeeding age. For instance, age

20 years is meant here to apply to the group of individuals between 20 years and 21 years of age; hence for the 20-year value the metabolism corresponding to the age point 20.5 years on the graph is given. For the younger ages, where the mean curve shows a metabolism rapidly changing with age, it was felt desirable to evaluate the metabolism for intervals of half a year at certain points. These values are given in table 2a. In this table, where no value is given for the half year, the value corresponding to the last whole year is to be used. Wherever the subdivision of the age is made into half years, the value corresponding to the whole year will also change. In table 2, for instance, age 8 years is intended to be

TABLE 2A

Basal metabolism, mean calories per square meter (DuBois) per hour. Supplementary table, smoothed values for age last birthday to half years for certain ages below twenty years. Where no value is given for the half year, the value corresponding to the last whole year is to be used.

AGE LAST BIRTHDAY	MEAN CALORIES PER SQUARE METER PER HOUR		AGE LAST BIRTHDAY	MEAN CALORIES PER SQUARE METER PER HOUR	
	Males	Females		Males	Females
year and half year			*year and half year*		
6	53.0	50.6	13	46.5	42.9
6½		50.2	13½		42.1
7	52.4	49.1	14	46.4	41.4
7½		47.8	14½		40.7
8	51.8	47.0	15	46.1	40.1
8½	51.2	46.5	15½		39.4
9	50.5	46.1	16	45.7	38.8
9½	49.4		16½	45.3	38.3
10	48.5	45.7	17	44.8	37.8
10½	47.7		17½	44.0	37.4
11	47.2	45.3	18	43.3	37.0
11½		44.8	18½	42.7	
12	46.8	44.3	19	42.3	36.6
12½		43.6	19½	42.0	

applied to individuals between 8 and 9 years of age, and therefore the metabolic value, which is given, corresponds to the point 8.5 years. In table 2a, age 8 years is intended to refer to individuals between 8 and 8.5 years of age; therefore, the metabolic value was read from the graph at the age point 8.25 years.

After the curves for the means were drawn, the goodness of fit of the curve was tested as follows: The difference between the observed mean at each age and the value given by the fitted curve at the corresponding age was expressed as a ratio to the standard deviation of this mean. For this purpose the standard deviation of the mean was calculated from the

smoothed instead of the observed standard deviation, as the former is the more reliable. The formula for the standard deviation of the mean, under these circumstances, is given by $\sigma_m = \frac{\sigma_s}{\sqrt{n}}$ where σ_m is the standard deviation of the mean, σ_s is the value of the standard deviation of the distribution given by the smoothed curve, and n is the number of individuals used in the calculation of the mean. The more usual formula, using $n-1$ instead of n in the denominator, is appropriate when only a single observed standard deviation is available for the distribution. Now if the observed means are distributed about the smoothed curve as random samples from a set of true values represented by the curve, the distribu-

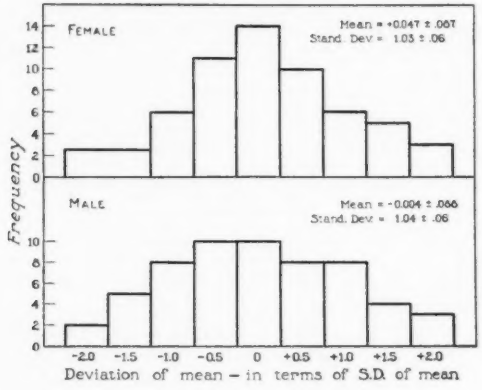

Fig. 2. Test of goodness of fit of mean curves: distribution of deviations of the means from the smoothed curve, each deviation expressed in ratio to the standard deviation of the pertinent mean.

tion of the ratios of the deviation of the means from the curve to the standard deviations of the means should be Gaussian; the mean of these ratios should have the value zero, and the standard deviation of their distribution should have the value unity. This is a comprehensive test of the goodness of fit of the smoothed curve. If the observed means are poorly balanced above and below the smooth curve, the mean of the ratios will differ significantly from zero, either positively or negatively. If the curve does not follow the means sufficiently closely, that is, if the means are scattered more widely about the curve than can be expected as a matter of chance, the standard deviation of the distribution will be significantly greater than unity. If the curve follows the means too closely, that is, if the curve has not been smoothed sufficiently and has been drawn

to follow the points very rigidly,[3] the standard deviation will be significantly less than unity. Thus we have each of the possible deviations from a good fit tested specifically. In figure 2 are shown the results of this test applied to both the male and the female curve of means. We see that the criteria for a good fit are amply met. The values for the

TABLE 3

Comparison of the Mayo Foundation standard with various bodies of data

SERIES, AUTHORS	TOTAL NUMBER	AGE — RANGE	NUMBER ABOVE M.F. STANDARD	NUMBER BELOW M.F. STANDARD	MEAN DIFFERENCE OBSERVED—M.F. STANDARD		STANDARD DEVIATION DIFFERENCE FROM M.F. STANDARD CORRECTED FOR LEVEL	
					Calories per square meter per hour	Per cent of standard mean	Calories per square meter per hour	Per cent of standard mean
Females								
		years						
Mayo Foundation	828	6–70	405	423	0.0	0.0	2.6	6.9
Harris and Benedict (4)	103	15–74	44	59	−0.4	−1.0	2.8	7.8
Magnus-Levy and Falk (10)	7	7–14	5	2	+1.1	+2.5	2.6	5.8
Magnus-Levy and Falk (9)	14	17–40	13	1	+4.7	+13.0	3.1	8.5
Palmer, Means, and Gamble (13)	7	20–24	3	4	−1.0	−2.9	2.3	6.4
Stark (15)	64	17–20	6	58	−3.3	−8.9	2.6	6.9
Males								
Mayo Foundation	639	6–64	315	324	0.0	0.0	2.9	6.7
Bierring (1)	133	7–19	18	115	−3.3	−7.0	2.7	5.7
DuBois (boy scouts) (3)	8	12–14	7	1	+2.8	+6.1	2.4	5.2
Harris and Benedict (5)	136	16–63	29	107	−1.9	−4.7	2.4	6.0
Harris and Benedict (6)	31	19–44	7	24	−1.6	−3.9	2.7	6.7
Magnus-Levy and Falk (11)	15	6–17	9	6	+1.3	+2.7	3.4	7.1
Magnus-Levy and Falk (7)	13	7–17	6	7	+0.4	+0.8	3.3	6.9
Magnus-Levy and Falk (8)	10	22–56	6	4	+0.3	+0.7	1.9	4.8
Palmer, Means, and Gamble (12)	8	25–32	0	8	−2.7	−6.8	1.2	3.0
Smith (14)	16	19–29	8	8	−0.1	−0.2	1.5	3.6
Stark (16)	62	16–20	4	58	−4.5	−10.3	2.3	5.4

means of these distributions are zero and for the standard deviations unity, well within significant limits.

We consider therefore that the curves represent well the means of the

[3] If the curve were expressed algebraically, this would be equivalent to the situation where too many constants had been employed in the function for the curve.

metabolism expressed as calories per square meter per hour at the various ages for this series of data. The use in connection with these values of the determination of the variability of metabolism measured as standard deviation will be discussed in what follows.

A comparison of the Mayo Foundation Standard with some of the various bodies of data collected by other authors has been made and the results are summarized in table 3. We have not attempted to include in this table all published series of data, but only made the necessary calculations for a number of representative ones. The table presents the comparisons practically as does Stark (17) and gives in addition some other summary facts. The means of the differences between observed values and the Mayo Foundation Standard values, as calories per square meter per hour, are given in the fifth column and in the next column these are given as a per cent of the mean of the standard values. Where the mean difference is positive it signifies that on the average the observed data are higher than the Standard, and when negative, it signifies that they are lower. This gives an average measure of the difference in level of the data examined and the Mayo Foundation Standard. In the next to the last column is given the standard deviation of the differences of the observed values from those given by the standard curve after the latter has been corrected by a constant equal to the mean difference in calories per square meter per hour.[4] In the last column this is expressed as a per cent of the mean of the standard values. These measure the scatter of the observations about the Mayo Foundation Standard, the latter raised or lowered so as to make the standard mean equal to the observed mean; essentially it reflects here the degree to which the variation with age in the observed data agrees with that represented by the Mayo Foundation Standard curve.

One may observe in table 3 that there is a significant difference of level among various data collected by different workers. On the other hand if the difference of level is adjusted, the standard deviation is generally about as small as that shown by comparing these data with standards built specifically to fit the data themselves. From this we may conclude that the age changes shown by these various bodies of data are in general agreement with those depicted by the Mayo Foundation Standards.

It is an important matter to discover the source of the differences in general level of metabolism found by different workers, but it would be futile to attempt to do this as part of this presentation. However, a few pertinent observations may be made. In various studies there has been

[4] This figure can be easily obtained from the mean and the mean square of the differences from the uncorrected standard by the relation $\sigma = \sqrt{mS - M^2}$ where mS is the mean of the squares of the observed differences, and M is the mean of these differences.

a consistent tendency to select the lowest obtainable determination as the most representative. The amount of selection has varied; in some instances it has been extreme. We believe that this accounts at least in part for the fact that most observations fall below ours, since we have regularly accepted the first determination, as has been explained previously. Probably there are also other factors involved, such as race, regional differences, and climate, as well as differences arising from the variation of instruments used; before the precise allocation of these can be attempted, some definite determination of the effect of selection will have to be made.

The purpose of determining the mean value of the basal metabolism of normal persons, so far as clinical practice is concerned, is to judge whether any particular individual differs from the expected mean so much that he may be considered probably abnormal. In usual clinical practice the difference of a particular observation, from the normal standard is expressed as a percentage of the standard, and this value is termed the "basal metabolic rate" or B.M.R. A B.M.R. of + 20 per cent, for instance, indicates that the individual has a basal rate 20 per cent above the average value found as normal for persons of the same sex and physical build. Now what is wanted is not this percentage figure as such, but rather the measure of the variability of normals around this mean, in order to gauge whether an observed individual is either probably normal or probably abnormal in respect to metabolism. Thus it has been the common practice to make the rough assumption that the significant normal range of variability is somewhere between ±10 per cent and ±15 per cent on the B.M.R. scale.

A more direct and precise measure corresponding to this concept would be a quantity that gives the definite fraction of normal individuals that have as large a discrepancy from the normal average, as does the person examined. For instance, if we can say that a given deviation from the average is of such magnitude that either 1 in 10, 1 in 100, or 1 in 1000 normal persons have so large a deviation, we have a definite quantitative basis of comparison for estimating the probability that that person is abnormal. In this paper we present a nomogram which gives, in addition to the usual B.M.R. reading, the probability of normality in the sense just described. The establishment of the probability scale was based on the "normal" or Gaussian curve, the integral of which has been conveniently tabulated. Given the mean (calories per square meter per hour) and the standard deviation, at any age, the probability of a departure from the mean, which is as large as any given amount, can be read off from these tabulations, if one may assume that the distribution of departures from the mean metabolism is sensibly Gaussian. We have studied the distribution of the variations of metabolism of normal individuals and found that

the differences observed from the average metabolism may be considered Gaussian. Evidence for this will be more fully presented in a separate study of the variability of metabolism in a forthcoming paper.

For the purposes of making the nomogram simple and easy to use,

TABLE 4

Standard values for calories per square meter per hour used at various ages as of last birthday in construction of nomogram

MALES		FEMALES	
Age last birthday	Mean	Age last birthday	Mean
years		*years*	
6	53.00	6	50.62
7	52.45	6½	50.23
8	51.78	7	49.12
8½	51.20	7½	47.84
9	50.54	8	47.00
9½	49.42	8½	46.50
10	48.50	9–10	45.90
10½	47.71	11	45.26
11	47.18	11½	44.80
12	46.75	12	44.28
13–15	46.35	12½	43.58
16	45.72	13	42.90
16½	45.30	13½	42.10
17	44.80	14	41.45
17½	44.03	14½	40.74
18	43.25	15	40.10
18½	42.70	15½	39.40
19	42.32	16	38.85
19½	42.00	16½	38.30
20–21	41.43	17	37.82
22–23	40.82	17½	37.40
24–27	40.24	18–19	36.74
28–29	39.81	20–24	36.18
30–34	39.34	25–44	35.70
35–39	38.68	45–49	34.94
40–44	38.00	50–54	33.96
45–49	37.37	55–59	33.18
50–54	36.73	60–64	32.61
55–59	36.10	65–69	32.30
60–64	35.48		
65–69	34.80*		

* Obtained by extrapolation.

certain approximations were made. Strictly, we should use at each age and for each sex separately, a specific mean and a specific standard deviation, since both of these vary with age and sex. So far as the mean is concerned, our approximations consisted in grouping certain ages in each

sex at points where the age change was small. The values for mean calories per square meter for the grouped ages used in the construction of the nomogram are given in table 4. So far as standard deviation is concerned, since this does not vary considerably, we grouped both sexes together and divided the age scale into two parts, that for individuals who are less than 20 years of age, and that for individuals who are 20 years or more of age. For the group of individuals who were less than 20 years of age, the value for standard deviation was taken as 3 calories

TABLE 5

Probability of specific deviations as given by the nomogram and as observed

DEVIATION FROM STANDARD MEAN, CALORIES PER SQUARE METER PER HOUR	PROBABILITY OF AS GREAT OR GREATER DEVIATION—PER 100 CASES					
	Age less than 20 years			Age 20 years or more		
	Normal curve (nomogram)	Observed		Normal curve (nomogram)	Observed	
		Male	Female		Male	Female
Observed greater than standard						
8	0.4	1.0 ±0.2	0.4 ±0.3	0.02	0.30 ±0.1	0.50 ±0.1
7	1.0	2.0 ±0.4	1.1 ±0.4	0.13	0.30 ±0.1	0.70 ±0.1
6	2.3	2.9 ±0.6	1.4 ±0.6	0.47	1.2 ±0.3	1.1 ±0.2
5	4.7	5.5 ±0.8	2.8 ±0.9	1.4	3.0 ±0.4	2.2 ±0.3
4	9.3	10.1 ±1.1	9.6 ±1.2	4.5	6.3 ±0.8	5.5 ±0.6
3	15.9	15.0 ±1.4	16.4 ±1.5	9.7	13.2 ±1.1	12.1 ±0.9
2	25.1	25.4 ±1.7	28.5 ±1.7	19.2	21.7 ±1.5	21.6 ±1.1
1	37.1	36.2 ±1.9	41.3 ±1.9	33.3	37.7 ±1.7	34.0 ±1.4
Observed less than standard						
1	37.1	40.7 ±1.9	34.9 ±1.9	33.3	35.2 ±1.7	36.6 ±1.4
2	25.1	28.0 ±1.7	22.1 ±1.7	19.2	20.2 ±1.5	20.8 ±1.1
3	15.9	19.2 ±1.4	14.6 ±1.5	9.7	12.0 ±1.1	8.4 ±0.9
4	9.3	11.4 ±1.1	9.3 ±1.2	4.5	7.2 ±0.8	3.7 ±0.6
5	4.7	5.5 ±0.8	5.0 ±0.9	1.4	3.0 ±0.4	1.1 ±0.3
6	2.3	2.6 ±0.6	2.5 ±0.6	0.47	0.9 ±0.3	0.2 ±0.2
7	1.0	1.6 ±0.4	1.4 ±0.4	0.13	0.0 ±0.1	0.2 ±0.1
8	0.4	0.0 ±0.2	0.7 ±0.3	0.02	0.0 ±0.1	0.2 ±0.1

per square meter per hour for both sexes; for the group of individuals who were 20 years or more of age, the value 2.5 was used. In the end we tested directly to determine whether these small approximations of mean and standard deviation had any significant effect in respect of the anticipated probabilities. Table 5 shows the comparison of the probabilities of departures of different magnitude for both age groups used, as between those given by the nomogram and those directly observed in the data. The probable error of the observed percentage is attached in each instance, and nowhere is the observed percentage significantly different from the

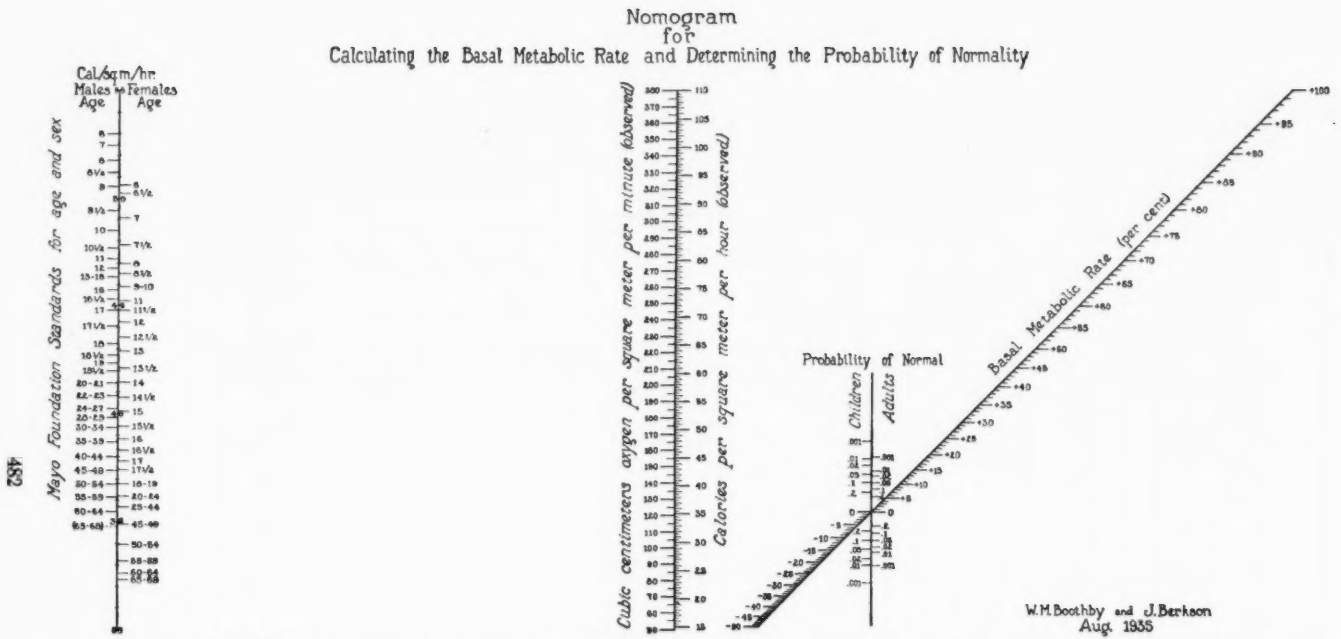

Fig. 3. *Directions for use of nomogram:* To determine the basal metabolic rate place a pin or needle on the observed calories per square meter (DuBois) or cubic centimeter oxygen per square meter (DuBois) and swing left hand end of ruler to the age of the subject for the appropriate sex on the left hand upright line—the Mayo Foundation Standards for age and sex. Read the B.M.R. in per cent on the right hand oblique line where the ruler crosses that line. In using the age standard, express age as of last birthday, as is the common custom of expressing age; observe half years when these are given on the nomogram. That is, a boy is 7 years old until he is 8 years old, and he is 8 years old until he is 8½ years old, and so forth. To determine the "probability of normal," read, before removing the ruler, the decimal on the short upright line. If it reads 0.02, that means that out of every 100 normal people two can be expected to have a B.M.R. as great (small) or greater (smaller) than observed in this particular subject. For purposes of this nomogram, "adult" refers to individuals 20 years or more of age. For example, a boy 9 years old whose observed metabolism is 55.5 calories per square meter per hour has a B.M.R. of +10 per cent and 5 normal boys of his age, out of 100, can be expected to have a B.M.R. of 10 per cent or greater. A man 32 years old whose metabolism is 44 calories per square meter per hour has a B.M.R. of +12 per cent and 2 similar normal individuals out of 100 can be expected to have a B.M.R. of 12 per cent or greater.

anticipated percentage when the customary criterion of significance of 3 times the probable error is used. In any case, the differences are generally in the order of a fraction of a per cent, and these are trivial in the practical use for which the nomogram is intended.

The nomogram (fig. 3) gives at one setting of the rule, the B.M.R. as usually expressed, in relation to the Mayo Foundation Standard, and also the "probability of normal." The method of using the nomogram is described in the legend.

So far as the figure reading "probability of normal" is concerned, it should be understood that this quantity gives the definite fraction of *normal* individuals who show the discrepancy at hand. In a broader sense than the one defined and used in connection with the application of the nomogram, the probability that a person examined is normal cannot be determined from the nomographic value alone. We have in mind here, that the clientele of a clinical practice is not equally distributed among normal and abnormal individuals, and that a comprehensive measure of the likelihood that a given individual is normal will have to include a weighting by the proportion of these two classes that actually obtains in the population dealt with. Also, it would have to include an extensive study of the variability as well as the mean value of the metabolism of abnormal individuals, classified into several groups, similar to the study made here for a group of normal persons. Such a study for the population with which The Mayo Clinic deals is under way and will be published in a succeeding paper.[5]

REFERENCES

(1) Bierring, E. The standard metabolism of boys. Levin and Munksgaard, Copenhagen, 1931.
(2) Boothby, W. M. and I. Sandiford. Laboratory manual of the technic of metabolic rate determinations. W. B. Saunders Company, Philadelphia, 1920.
(3) DuBois, E. Quoted by Harris and Benedict, 1919, p. 237.
(4) Harris, J. A. and F. G. Benedict. A biometric study of basal metabolism in man. Carnegie Institution, Washington, 1919, p. 44.
(5) Harris, J. A. and F. G. Benedict. Carnegie Inst., Washington, p. 40, 1919.
(6) Harris, J. A. and F. G. Benedict. Carnegie Inst., Washington, p. 234, 1919.
(7) Magnus-Levy and Falk. Quoted by Bierring, 1931, p. 13.
(8) Magnus-Levy and Falk. Quoted by Harris and Benedict, 1919, p. 233.
(9) Magnus-Levy and Falk. Quoted by Harris and Benedict, 1919, p. 236.
(10) Magnus-Levy and Falk. Quoted by Harris and Benedict, 1919, p. 238.
(11) Magnus-Levy and Falk. Quoted by Harris and Benedict, 1919, p. 238.

[5] An example of the relevant methodology for such a study in connection with the laboratory estimation of gastric acidity is to be found in F. R. Vanzant and W. C. Alvarez. Am. J. Digest. Dis. and Nutrition **2**: 466, 1935.

(12) PALMER, MEANS AND GAMBLE. Quoted by HARRIS AND BENEDICT, 1919, p. 231.
(13) PALMER, MEANS AND GAMBLE. Quoted by HARRIS AND BENEDICT, 1919, p. 231.
(14) SMITH, H. M. Quoted by HARRIS AND BENEDICT, 1919, p. 244.
(15) STARK, M. E. Trans. Wisc. Acad. Sci. **27**: 264, 1932.
(16) STARK, M. E. This Journal **111**: 631, 1935.
(17) STARK, M. E. This Journal **111**: 635, 1935.

STUDIES OF THE ENERGY OF METABOLISM OF NORMAL INDIVIDUALS. A COMPARISON OF THE ESTIMATION OF BASAL METABOLISM FROM (1) A LINEAR FORMULA AND (2) "SURFACE AREA"

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In the preceding paper, the energy of metabolism was considered in relation to the surface area, the latter estimated from stature and body weight, by the formula of DuBois and DuBois. When we standardize metabolism in this way, it is equivalent to using as the standard of metabolism at any age, a value obtained by the exponential formula:

$$h_a = M_a \times 0.007184 \times W^{0.425} \times S^{0.752}$$

where h_a represents the predicted calories per hour specific for age a; M_a represents the mean calories per square meter per hour used as standard at age a; W represents the body weight in kilograms, and S is stature in centimeters.

Instead of this formula for predicting total metabolism, others have been advanced, as either equally good or better. Notably, Harris and Benedict (1), in their exhaustive biometric analysis of basal metabolism, have advanced the linear formula:

$$h = a + b\,W + c\,S + d\,A$$

in which h represents the calories per 24 hours; S and W represent stature and weight as before and A represents age in years; while a, b, c, and d are constants determined by statistical methods.

In view of the extensiveness of the analysis made by Harris and Benedict and because of the wide currency given their conclusions, before we erected our work on the basis of the surface-area (DuBois) formulation, we examined minutely and critically the merits of the linear formulation. This was done by using both the data given in the treatise of Harris and Benedict, and all our own data. It is the purpose of this paper to present the results of that examination.

The main emphasis in the analysis of Harris and Benedict was an accuracy of prediction, and we shall similarly center our discussion around that consideration. It will be appropriate to make several preliminary observations with respect to this. The departure of observed metabolism from

values predicted by a formula may be regarded as arising from three sources: 1, the variability of any particular normal individual's metabolism, when repeated observations are made under standard conditions, around the mean metabolism of that individual; 2, the variability of the mean metabolism of different normal individuals around the mean metabolism of all the individuals in the same specific class (specified for sex and age, stature and weight); and 3, the "errors" of the formula used, that is, the extra variability introduced by the fact that the formula predicts only approximately the mean values for the appropriate specific class of individuals. Now the part of the total variation comprised in (1) and (2) is in no wise related to the formula used, but only that part which is contained in (3) has to do with the efficiency of the formula. The variability of repeated determinations on the same individual, which is what is comprised in (1), has been found to average about 2.5 calories per hour, measured as the standard deviation. Taken together with the variability of different individuals, which is what is comprised in (2), it amounts to about 3 calories in a large representative series. These figures are approximate but they will serve to give the order of the accuracy of prediction that can be expected in a "perfect" formula. The differences in the accuracy of prediction that will result from differences in the efficiency of alternative formulas used, are bound to be small. One may see this clearly if one considers a simple equation which gives the relationship of the total variability to several variabilities of which it is composed, if each is measured as the standard deviation; this equation is as follows:

$$\sigma_t = \sqrt{\sigma_i^2 + \sigma_I^2 + \sigma_f^2}$$

σ_t is the total variability, σ_i is the intra-individual variability, σ_I is the inter-individual variability, and σ_f is the variability assignable to the formula. It is a consequence of this equation that if the constituent variabilities are different in magnitude one from the other, the total variability will be practically equal to the largest constituent. From this, it follows that if any one constituent is not as large as the sum of the others, it can affect the total variability only slightly. This can be made clear by an hypothetical example. Suppose σ_i and σ_I are each equal to 1, and σ_f is equal to 0.1. Then σ_t, the total variability, is equal to $\sqrt{2.01}$ or 1.42. If σ_f is now raised to 0.5, the total variability is equal to $\sqrt{2.25}$ or 1.50. In this hypothetical example, σ_f, the variability measuring the "error" of the formula, has been raised 400 per cent, and the effect on the total variability is to raise it only 6 per cent. Here one may see the difficulty of comparing formulas by measuring the average error of prediction. If several types of formulas are fitted to the same set of metabolic observations, each by consistent statistical methods, the predictions of all are likely to agree

reasonably well with the data to which they were fitted.[1] In view of what has been pointed out regarding the necessary variation of predicted from observed metabolism, which arises from the intra-individual and inter-individual variability of metabolism, the differences between the accuracy of prediction of various formulas that are fitted by consistent statistical methods will be minute. These preliminary remarks are made with the purpose of guiding an understanding of the analysis that follows.

Harris and Benedict used the criterion of accuracy of prediction for testing the relative validity of the linear and surface-area method of prediction. We therefore first applied the same method to our own data. For predicting from surface area, we used the mean calories per square meter per hour given by the Mayo Foundation Standard (table 2 in the preceding paper). For the linear method, it was necessary to obtain comparable linear formulas which would apply to these same data. Since, obviously, the data are not linear over the entire age range, it was necessary to divide them into groups in which this would be at least approximately true. Accordingly, they were divided into three age groups, 6 to 12 years, 12 to 20 years, 20 years and over. In the last group the age corresponds roughly to the age range in the data of Harris and Benedict. We then fitted linear equations by least squares to each of the six groups of data comprised by the three age divisions of the males and females. It need hardly be pointed out that dividing the data into separate age groups before fitting the linear equations makes these equations accord more closely with the data than if such a preliminary division had not been made. The agreement between the predictions by the various equations tested, and the observations, was measured as the root-mean-square[2] of the differences between prediction of calories per hour, and observations of this quantity for each individual. The root-mean-square or standard deviation from predicted values will here be measured in calories. In the preceding paper, variation was measured as calories per square meter per hour, since the Mayo Foundation Standards with which they dealt are so expressed; but in the discussion in which we are here engaged we are to compare our results with a linear equation which predicts total calories, hence, for comparison, the Mayo Foundation Standards are also used as equations for predicting total calories, and differences from such predictions are likewise measured in these units. For the Mayo Foundation data, the results are shown in table 1. An examination of this table shows that there is no significant difference between the agreement of prediction, as between the linear

[1] Unless an extremely inappropriate type of equation is used, which practically will seldom be the case.

[2] This term which was used by Harris and Benedict, is synonymous with what has been referred to in our discussion as the standard deviation of observed from predicted values.

and surface-area formulation. In fact, the results are practically identical and both give about the best agreement that we can expect, considering the inherent variability of the observations.

A similar analysis was then made of the data presented in the treatise of Harris and Benedict. This was made possible by the fact that the authors presented their results in detail, and all the original observations were made available by them in admirably complete tabulations. For the linear equations we used, naturally, those given by Harris and Benedict. For the predictions by the surface-area formulation, we required values for the mean calories per square meter for specific ages. To obtain these, we calculated the means from their observations, and after plotting

TABLE 1

The Mayo Clinic Series: root-mean-square deviations, observed minus calculated calories per hour

AGE	MALES					FEMALES				
	Number	Linear equation		Surface-area method		Number	Linear equation		Surface-area method	
		Calories	Per cent of mean	Calories	Per cent of mean		Calories	Per cent of mean	Calories	Per cent of mean
years										
6–11	141	3.02	6.2	3.01	6.2	136	2.87	6.3	2.95	6.4
12–19	166	4.78	7.2	4.78	7.2	145	4.22	7.3	4.32	7.5
20+	332	4.70	6.9	4.55	6.7	547	3.94	7.0	3.93	6.9

Surface-area equation:

$$h = (\text{Ca. p. sq. m. p. hr.}) \times 0.007184 \times W^{0.425} \times S^{0.725}$$

Linear equations:

Male: 6–11 years. $h = 12.391 + 0.777\,W + 0.156\,S - 0.461\,A$

12–19 years. $h = 13.437 + 0.728\,W + 0.198\,S - 0.888\,A$

20+ years. $h = 4.071 + 0.452\,W + 0.256\,S - 0.250\,A$

Female: 6–11 years. $h = 15.192 + 0.706\,W + 0.098\,S - 0.052\,A$

12–19 years. $h = 7.516 + 0.420\,W + 0.336\,S - 1.503\,A$

20+ years. $h = 1.137 + 0.382\,W + 0.230\,S - 0.109\,A$

them, drew a smooth curve to the points. The standard values for calories per square meter per 24 hours, which were obtained in this way, are shown in figure 1 and tabulated in table 2. We also had available calculations, by Harris and Benedict, of the heat production in relation to surface area in broad age groups, presented in an early part of their discussion (p. 118). Both sets of values, the ones obtained by our own smoothing, and theirs, were used, but since there was no appreciable difference in results, the values which they gave were the ones used in the tabulations of precision of prediction presented in this paper. The data of Harris and Benedict were analyzed this way for the three groups which they gave; their main adult male series (136), their main adult female series (103) and a separate

group of 31 adult males (p. 234), the last particularly appropriate, because they were made available to Harris and Benedict after their equations had been evolved from their main series. The results are shown in table 3. An examination of this table discloses that here again, as in the case of the

TABLE 2

Data of Harris and Benedict, smoothed values (by present authors) for standard calories per square meter per twenty-four hours

AGE	CALORIES PER SQUARE METER PER TWENTY-FOUR HOURS		AGE	CALORIES PER SQUARE METER PER TWENTY-FOUR HOURS	
	Males	Females		Males	Females
years			*years*		
15		1,028	45	848	846
16	1,030	990	46	847	842
17	1,012	942	47	846	838
18	997	914	48	844	833
19	979	894	49	843	829
20	961	880	50	842	824
21	947	874	51	840	819
22	934	871	52	839	814
23	924	870	53	838	809
24	920	869	54	836	804
25	920	868	55	834	799
26	920	867	56	833	794
27	920	866	57	832	789
28	920	866	58	831	783
29	920	865	59	830	777
30	919	864	60	829	771
31	916	864	61	828	765
32	909	863	62	826	759
33	892	862	63	825	753
34	873	861	64	823	747
35	860	860	65	822	741
36	852	860	66		735
37	849	859	67		730
38	849	858	68		722
39	849	858	69		718
40	849	857	70		717
41	849	857	71		712
42	849	856	72		711
43	849	853	73		709
44	849	850	74		708

Mayo Foundation observations, there is practically no difference in the precision of prediction of the two formulas. Such slight differences as exist are preponderantly in favor of the surface-area formulation.

These numerical results are at variance with the results of Harris and

Benedict, who concluded that the linear formulas gave better predictions (table 75, p. 192). A careful examination of their work disclosed that the source of discrepancy resided in a difference in methods used for predicting from the surface-area formula. They utilized for this purpose a single value for each sex for the calories per square meter per 24 hours at all ages, namely, the mean value for the entire series (p. 178), whereas we used a

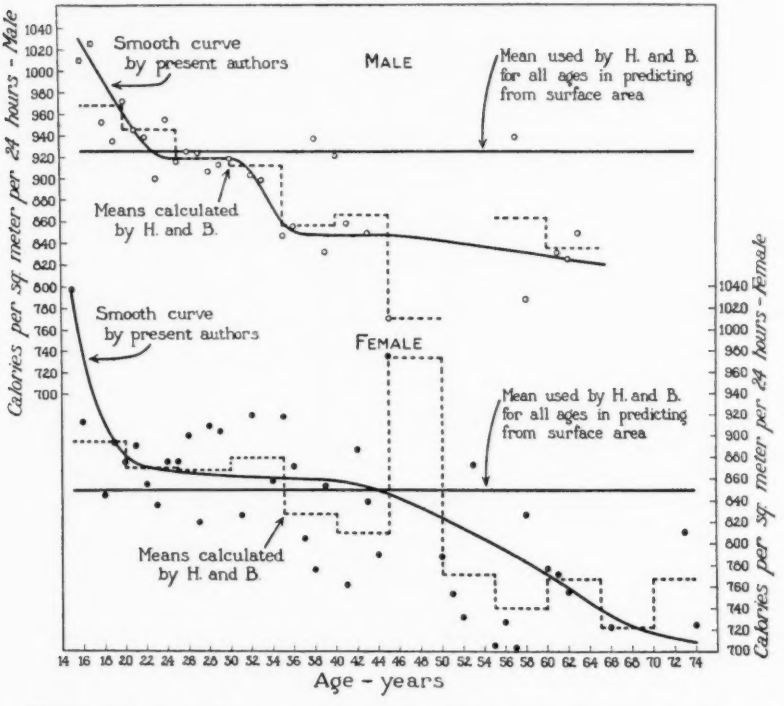
Smooth curve by present authors
MALE
Mean used by H. and B. for all ages in predicting from surface area
Means calculated by H. and B.
Smooth curve by present authors
FEMALE
Mean used by H. and B. for all ages in predicting from surface area
Means calculated by H. and B.
Calories per sq. meter per 24 hours - Male
Calories per sq. meter per 24 hours - Female
Age - years

Fig. 1. Data from Harris and Benedict: Mean calories per square meter per 24 hours. Observed values, values calculated by Harris and Benedict, curve smoothed by present authors, and value used by Harris and Benedict in predicting heat production from surface area.

value specific for age. If the surface-area formula is used without modification for age, their conclusion that it will not predict metabolism with as great precision as a linear formula in which age is included as one variable, is correct. However, if age is included in both formulas, we must conclude that both formulations give about equally good predictions, when an average deviation for the entire series is used as criterion.

We do not conclude from this that both formulations are equally appro-

priate or useful. On the contrary, we hold that the surface-area formulation is distinctly the preferable one. The fact is, that the criterion of an average measurement of the deviation of predicted from observed values, is not a crucial one under the conditions studied here. Under the conditions of considerable variability inherent in the observations of metabolism, it would require a very great many more observations than are available, to determine whether differences between the accuracy of prediction, measured by this criterion, were statistically significant. It is notable that Harris and Benedict attached no determination of the probable error to their estimation of differences of prediction accuracy.

Our judgment of the relative value of the linear and the surface-area formulation must be made on the implications of these formulations, and their relative values as instruments of biologic investigation. Now the constants of the formula of Harris and Benedict or of our own linear formulas cannot be given biologic interpretation. The constants in the

TABLE 3

Data from Harris and Benedict, root-mean-square observed minus calculated calories per twenty-four hours

SERIES	NUMBER	LINEAR EQUATION		SURFACE-AREA METHOD	
		Calories	Per cent of mean	Calories	Per cent of mean
Male, main series	136	101.7	6.2	102.7	6.3
Female, main series	103	106.3	7.9	104.8	7.1
Male, supplementary series	31	115.3	7.0	113.6	6.9

Harris and Benedict equations for males and females have little resemblance; indeed, the a constant for the females is 10 times that for the similar constant for the males. This has the effect of predicting for certain statures and body weights a higher metabolism for females than for males of the same age, as illustrated in table 4. This is an implication which has not been advanced independently as an observation, and is contrary to general impression and the findings in our own series of data. It is probably attributable to the circumstance that a small number of cases (statistically) were used in fitting the equations, and to the fact that the same age period in years for which both male and female equations were fitted are not physiologically equivalent in both sexes. The further implication of a linear formula that the decrease of metabolism with age is the same at each age is again contrary to observation. In a succeeding paper we will discuss the change of metabolism measured as calories per square meter, with age, for both sexes, where it will be found that the metabolic changes are characteristic in particular age periods. It is diffi-

cult to conceive how these or similar studies could be pursued effectively in terms of the linear formulation. Finally, as Harris and Benedict emphasize, their method of formulation cannot be extrapolated for use in children and it would be necessary, as found in the study of the applicability of the linear formulation to our own data, to have a series of formulas with different constants for various age and sex groups.

In this discussion we have viewed the relation of surface area to metabolism as an equational formulation. The surface area is here the shorthand expression for the height-weight formula of DuBois. Whether or not surface area as such is related to metabolism by affecting the rate of cooling of the body or through some other physical process would be impossible to determine conclusively from such observations as ours. It is possible to hold that the height-weight formula gives a fair statistical measure of the amount of energy consuming tissue in the body, and that this is the

TABLE 4

Illustration of instances in which for the same stature, weight and age, the equations of Harris and Benedict predict higher metabolism for females than males

AGE	STATURE	WEIGHT	CALORIES PER TWENTY-FOUR HOURS PREDICTED BY HARRIS AND BENEDICT EQUATION	
			Males	Females
years	*cm.*	*kgm.*		
21	137	45	1,229	1,241
30	143	46	1,212	1,220
40	145	47	1,168	1,186
50	146	53	1,188	1,199
50	135	40	955	1,055

physical basis of the relation of that formula to metabolism. All we can say here is that the hypothesis that when other factors are equal, the normal metabolism is proportional to surface area, when this is measured by the formula of DuBois, is consistent with all the observations we have examined.

We can add that other calculations which we made, but which do not appear worth while to present in detail, would seem to support this view. At the expense of great arithmetic labor, we fitted formulas of the same exponential type[3] as the DuBois formula, by least squares allowing the constants to take the values which would give good fits, instead of using those given in the surface-area formula of DuBois. It was our hope that we might in this way evaluate parameters which taken with stature and

[3] That is, where $h = k S^m W^n$, in which k, m, n are parameters to be evaluated by least squares, and h, S, W are the observed values of heat production, stature and weight.

weight would give better predictions of metabolism. Constants obtained in this way would be entirely independent of any *a priori* considerations and the method is similar to that used by Harris and Benedict for obtaining the constants of their linear type of formula. Such a successful combination, if found, might be interpreted as a formula either for "effective surface area" or for "active protoplasmic mass." We were disappointed that there was no consistency in the exponential parameters so evaluated at the various ages. It was interesting that the average value of these constants for arbitrarily selected age groups was not significantly different from the constants used by DuBois and obtained by him in his investigations of surface area. Further, taking into account the fact the formulas were applied to the data from which they were derived, they did not give impressively better predictions even at particular ages, than did the surface-area formula. Here again, therefore, we found that it was impossible to improve on the surface-area formulation.

We further attempted to investigate statistically whether a correction to the surface-area formula could be obtained to take account of the build of the body. Such attempts have been made by using indices of stature and weight derived from *a priori* considerations. We approached the question objectively on the following basis. If there is a difference in the proportionality of heat production and surface area for persons of different builds one can expect that there would be a consistent correlation between heat production per square meter, and stature or weight or both. If the calories per square meter are plotted against height or against weight or surface area one would expect a consistent variation from the average with high and low values of the body measure. From this correlation, a correction factor could be calculated. When we made such a trial, no significant correlation was discernible. This is not to conclude that the shape or build of the body does not affect the metabolism independently of surface area, but only that in the light of the variation inherent in our data, and within the range of variability of build represented in our normal series, no such effect could be ascertained statistically.

Discussion. A comparison has been made of the method of standardizing normal basal metabolism by means of a linear equation in weight, stature and age proposed by Harris and Benedict, and the method of using the height-weight formula of DuBois and DuBois for surface area, in conjunction with a specific constant for each age. The accuracy of the prediction of observed values, using both methods, was compared using our data and the data of Harris and Benedict. It was pointed out that the variation of predicted from observed values of metabolism is made up of the intra-individual and inter-individual variation of metabolism as well as the inaccuracy of the formula itself. In view of the considerable variation of metabolism among individuals of the same sex, age, stature

and weight, the variations of observed metabolism from values predicted by formulas employing these variables are also bound to be considerable. As a corollary, the differences between the precision of alternative formulas fitted by consistent methods and using the same variables will be small, since the inaccuracy of the formula contributes only a small part to the total discrepancy between the predicted and observed values. Exceedingly large numbers of observations would be required to determine conclusively that such small differences are significant; hence, the difficulty of distinguishing the relative merits of alternative formulas by utilizing the accuracy of predictions as a criterion.

We found no significant differences between the precision of prediction of heat production of the linear and "surface-area" formulas, either in the case of our data or those of Harris and Benedict. The contrary conclusion of Harris and Benedict was due to the omission by them of the age variable in applying the surface-area formula.

Our conclusions favored the surface-area formulation, because of the consistency of its implications with observations, and the relatively greater simplicity with which it can be used in the study of the change of metabolism with age or other variables. In the use of the surface-area formulation specific for age, the relationship of metabolism to age can be studied independently of its relation to stature and weight, which is an important methodological advantage. If we use furthermore, at each age, a function of stature and weight (surface area) to which metabolism is proportional, we can use the simple ratio of the metabolism to this function (calories per square meter) to express succinctly the relationship of metabolism to stature and weight at every age specifically. It is from these formal considerations that the surface-area formulation derives its advantages as an instrument of investigation.

REFERENCE

(1) Harris, J. A. and F. G. Benedict. A biometric study of basal metabolism in man. Washington, Carnegie Inst., 1919, 266 pp.